



US009101745B2

(12) **United States Patent**
Coles et al.

(10) **Patent No.:** US 9,101,745 B2
(45) **Date of Patent:** Aug. 11, 2015

(54) **SONOCHEMICAL INDUCTION OF ABCA1 EXPRESSION AND COMPOSITIONS THEREFOR**

(71) Applicant: **SONOGENE, LLC**, Glen Ellyn, IL (US)

(72) Inventors: **Eric Coles**, Glen Ellyn, IL (US); **Michael Davidson**, Highland Park, IL (US)

(73) Assignee: **SONOGENE LLC**, Glen Ellyn, IL (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 62 days.

(21) Appl. No.: **13/826,066**

(22) Filed: **Mar. 14, 2013**

(65) **Prior Publication Data**

US 2014/0276363 A1 Sep. 18, 2014

(51) **Int. Cl.**

A61M 37/00 (2006.01)
A61K 31/7088 (2006.01)
A61K 45/06 (2006.01)
A61K 49/22 (2006.01)
A61K 41/00 (2006.01)
A61K 48/00 (2006.01)
A61K 31/713 (2006.01)
C12N 15/00 (2006.01)

(52) **U.S. Cl.**

CPC **A61M 37/0092** (2013.01); **A61K 31/7088** (2013.01); **A61K 31/713** (2013.01); **A61K 41/0028** (2013.01); **A61K 45/06** (2013.01); **A61K 48/00** (2013.01); **C12N 15/00** (2013.01)

(58) **Field of Classification Search**

CPC A61K 41/0028; A61M 37/0092
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,190,766 A	3/1993	Ishihara et al.
5,498,421 A	3/1996	Grinstaff et al.
5,542,935 A	8/1996	Unger et al.
5,558,092 A	9/1996	Unger et al.
5,580,575 A	12/1996	Unger et al.
5,770,222 A	6/1998	Unger et al.
5,849,727 A	12/1998	Porter et al.
5,961,459 A	10/1999	Kaul et al.
6,048,903 A	4/2000	Toppo
6,066,123 A	5/2000	Li et al.
6,068,857 A	5/2000	Weitschies et al.
6,071,495 A	6/2000	Unger et al.
6,117,858 A	9/2000	Porter et al.
6,120,751 A	9/2000	Unger
RE36,939 E	10/2000	Tachibana et al.
6,135,976 A	10/2000	Tachibana et al.
6,139,819 A	10/2000	Unger et al.
6,265,387 B1	7/2001	Wolff et al.
6,575,956 B1	6/2003	Brisken et al.
7,211,248 B2 *	5/2007	Davidson
7,351,535 B2	4/2008	Lawn et al.

2001/0008758 A1 7/2001 McHale et al.
2001/0009904 A1 7/2001 Wolff et al.
2002/0165191 A1 11/2002 Moonen
2004/0266663 A1 12/2004 Schwartz et al.

FOREIGN PATENT DOCUMENTS

CN	1056124	11/1995
WO	0042988 A1	7/2000

OTHER PUBLICATIONS

Vaisman et al. (J. Clin. Invest. 2001; 1008: 303-309).
Attie, A.D. et al., Pivotal Role of ABCA1 in Reverse Cholesterol Transport Influencing HDL Levels and Susceptibility to Atherosclerosis, Journal of Lipid Research, vol. 42, 1717-1726 (2001).
Dong, C. et al., ABCA1 Single Nucleotide Polymorphisms: Snipping at the Pathogenesis of Atherosclerosis, Circulation Research, vol. 88, 855-857 (2001).
Oram, J.F. et al., ATP-Binding Cassette Cholesterol Transporters and Cardiovascular Disease, Circulation Research, vol. 99, 1031-1043 (2006).
Shohet, R.V. et al., Echocardiographic Destruction of Albumin Microbubbles Directs Gene Delivery to the Myocardium, Circulation, vol. 101, 2554-2556 (2000).
Zarubica, A. et al., Functional Implications of the Influence of ABCA1 on Lipid Microenvironment at the Plasma Membrane: a Biophysical Study, The FASEB Journal, vol. 23, 1775-1785 (2009).
Trompier, D. et al., Transition from Dimers to Higher Oligomeric Forms Occurs During the ATPase Cycle of the ABCA1 Transporter, Journal of Biological Chemistry, vol. 281, No. 29, 20283-20290 (2006).
He, Y. et al., Ultrasound Microbubble-Mediated Delivery of the siRNAs Targeting MDR1 Reduces Drug Resistance of Yolk Sac Carcinoma L2 Cells, Journal of Experimental & Clinical Cancer Research, vol. 30:104, 1-11 (2011).
Dijkmans, P.A. et al., Microbubbles and Ultrasound: From Diagnosis to Therapy, Eur. J. Echocardiography, vol. 5, 245-256 (2004).
Lawrie, A. et al., Microbubble-Enhanced Ultrasound for Vascular Gene Delivery, Gene Therapy, vol. 7, 2023-2027 (2000).
Kaufman, R.J., Overview of Vector Design for Mammalian Gene Expression, Molecular Biotechnology, vol. 16, 151-160 (2000).
Smith, B., et al., Anticancer Activity of the Cholesterol Exporter ABCA1 Gene, Cell Reports 2, 580-590 (2012).

(Continued)

Primary Examiner — Scott Long

(74) Attorney, Agent, or Firm — Olson & Cepuritis, Ltd.

(57) **ABSTRACT**

The present invention provides compositions useful for transfecting cells (e.g., liver cells) to express ABCA1. The compositions described herein comprise a pharmaceutically acceptable aqueous carrier containing sonochemically-active microspheres together with a plasmid DNA construct encoding an active form of ABCA1 and at least one promoter for the expression thereof. Preferably, the sonochemically-active microspheres comprise, consist essentially of, or consist of gas bubbles (e.g., a fluorocarbon gas, such as octafluoropropane) encapsulated within protein-containing or lipid-containing shells (e.g., human serum albumin shells). The microspheres are disruptable by exposure to ultrasonic acoustic energy to release the encapsulated gas.

(56)

References Cited**OTHER PUBLICATIONS**

- Duong, P.T. et al., Characterization and Properties of Pre β -HDL Particles Formed by ABCA1-Mediated Cellular Lipid Efflux to ApoA-1, *J. Lipid Res.* vol. 49(5), 1006-1014 (2008).
- Iatan, I. et al., Effect of ABCA1 Mutations on Risk for Myocardial Infarction, Clinical Trials and Their Interpretations, *Current Atherosclerosis Reports*, vol. 10, 413-426 (2008).
- Oram, J.F. et al., ATP-Binding Cassette Transporter A1: A Cell Cholesterol Exporter that Protects Against Cardiovascular Disease, *Physiol. Rev.* vol. 85, 1343-1372 (2005).
- Ohashi R. et al., Reverse Cholesterol Transport and Cholesterol Efflux in Atherosclerosis, *QJ Med* vol. 98, 845-856 (2005).
- Sporstol, M. et al., ABCA1, ABCG1 and SR-BI: Hormonal Regulation in Primary Rat Hepatocytes and Human Cell Lines, *BMC Molecular Biology* vol. 8 (5), 1-8 (2007).
- Yang, Y. et al., Suppression of ABCA1 by Unsaturated Fatty Acids Leads to Lipid Accumulation in HepG2 Cells, *Biochimie* vol. 92, 958-963 (2010).
- Liao, H. et al., Native LDL Upregulation of ATP-Binding Cassette Transporter-1 in Human Vascular Endothelial Cells, *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 22, 127-132 (2002).
- GenBank: OriGene TrueORF Data, *Homo sapiens* ATP-Binding Cassette, Sub-family A (ABC1) Member 1 (ABCA1), mRNA, NCBI Reference Sequence NM_005502.2.
- Skyba, D.M. et al., Direct In Vivo Visualization of Intravascular Destruction of Microbubbles by Ultrasound and Its Local Effects on Tissue, *Circulation* vol. 98, 290-293 (1998).
- Klibanov, A.L. et al., Targeted Delivery of Gas-Filled Microspheres, Contrast Agents for Ultrasound Imaging, *Advanced Drug Delivery Reviews*, vol. 37, 139-157 (1999).
- Miller, D.L. et al., Ultrasonic Enhancement of Gene Transfection in Murine Melanoma Tumors, *Ultrasound in Medicine & Biology*, vol. 25 (9), 1425-1430 (1999).
- Kim, H.J. et al., Ultrasound-Mediated Transfection of Mammalian Cells, *Human Gene Therapy* vol. 7, 1339-1346 (1996).
- Lawrie, A. et al., Ultrasound-Enhanced Transgene Expression in Vascular Cells Is Not Dependent Upon Cavitation-Induced Free Radicals, *Ultrasound in Med. & Biol.*, vol. 29 (10), 1453-1461 (2003).
- Shohet et al., Microbubbles Used to "Pop" New Gene Into the Heart, *Clinical Genetics*, vol. 58(4), 269 (2000).
- Manome, Y. et al., Ultrasound Facilitates Transduction of Naked Plasmid DNA into Colon Carcinoma Cells in Vitro and in Vivo, *Human Gene Therapy*, vol. 11, 1521-1528 (2000).
- Anwer, K. et al., Ultrasound Enhancement of Cationic Lipid-Mediated Gene Transfer to Primary Tumors Following Systemic Administration, *Gene Therapy*, vol. 7, 1833-1839 (2000).
- Tangirala, R.K. et al., Regression of Atherosclerosis Induced by Liver Directed Gene Transfer of Apolipoprotein A-I in Mice, *Circulation*, vol. 100, 1816-1822 (1999).
- Dansky, H.M. et al., High-Density Lipoprotein and Plaque Regression, The Good Cholesterol Gets Even Better, *Circulation*, vol. 100, 1762-1763 (1999).
- Tsukamoto, K. et al., Comparison of Human ApoA-1 Expression in Mouse Models of Atherosclerosis After Gene Transfer Using a Second Generation Adenovirus, *Journal of Lipid Research*, vol. 38, 1869-1876 (1997).
- Boisvert, W.A. et al., ApoA1 Reduces Free Cholesterol Accumulation in Atherosclerotic Lesions of ApoE-Deficient Mice Transplanted with ApoE-Expressing Macrophages, *Arterioscler Thromb Vasc Biol.* vol. 19, 525-530 (1999).
- Huber, P.E. et al., In Vitro and In Vivo Transfection of Plasmid DNA in the Dunning Prostate Tumor R3327-AT1 is Enhanced by Focused Ultrasound, *Gene Therapy*, vol. 7, 1516-1525 (2000).
- Lawrie, A. et al., Ultrasound Enhances Reporter Gene Expression After Transfection of Vascular Cells in Vitro, *Circulation*, vol. 99, 2617-2620 (1999).
- Dass C. et al., Apolipoprotein A-1, Phospholipid Vesicles and Cyclodextrins as Potential Anti-Atherosclerotic Drugs: Delivery, Pharmacokinetics, and Efficacy, *vol. 7*, 161-182 (2000).
- Nanjee, M.N. et al., Acute Effects of Intravenous Infusion of ApoA1/Phosphatidylcholine Discs on Plasma Lipoproteins in Humans, *Arterioscler Thromb Vasc. Biol.*, vol. 19, 979-989 (1999).
- Chomas, J.E. et al., Threshold of Fragmentation for Ultrasonic Contrast Agents, *Journal of Biomedical Optics*, vol. 6 (2), 141-150 (2001).
- Fechheimer, M. et al., Transfection of Mammalian Cells with Plasmid DNA by Scrape Loading and Sonication Loading, *Proc. Natl. Acad. Sci.*, vol. 84, 8463-8467 (1987).
- Product Insert for OPTISON Ultrasound Contrast Agent, "OPTISON" (Perflutren Protein—Type A Microspheres for Injection, USP, revised Jun. 2003).
- Reznik, N. et al., On the Acoustic Properties of Vaporized Submicron Perfluorocarbon Droplets, *Ultrasound in Med. & Biol.*, vol. 40 (6), 1379-1384 (2014).
- Sun, D. et al., MiR-26 Controls LXR-dependent Cholesterol Efflux by Targeting ABCA1 and ARL7, *FEBS Letters* 586, 1472-1479 (2012).
- Panje, C.M. et al., Ultrasound-Mediated Gene Delivery with Cationic Versus Neutral Microbubbles: Effect of DNA and Microbubble Dose on In Vivo Transfection Efficiency, *Theranostics* 2 (11), 1078-1091 (2012).
- Takahashi, M. et al., Spinal Gene Transfer Using Ultrasound and Microbubbles, *Journal of Controlled Release* 117, 267-272 (2007).
- Hernot, S. et al., Microbubbles in Ultrasound-Triggered Drug and Gene Delivery, *Advanced Drug Delivery Reviews* 60, 1153-1166 (2008).
- Guo, D.-P. et al., Ultrasound-Targeted Microbubble Destruction Improves the Low Density Lipoprotein Receptor Gene Expression in HepG2 Cells, *Biochemical and Biophysical Research Communications* 343, 470-474 (2006).

* cited by examiner

(SEQ ID NO: 1):

MACWPQLRLLLWKNLTFRQQTCQLLLEVAWPLFIFLILISVRLSYPPYEQHECHFPNKA
MPSAGTLPWVQGIICNANNPCFRYPTGEAPGVVGNFNKSIVARLFSDARRLLYSQKDT
SMKDMRKVLRTLQQIKSSSNLKLDQFLVDNETFSGFLYHNLSLPKSTVDKMLRADVILH
KVFLQGYQLHLSLCNGSKSEEMIQLGDQEVSCLCGLPREKLAACERVLRSNMDILKPIL
RTLNSTSPFPSKELAEATKTLHSLGTLAQELFSMRSWSDMRQEVMFVNNSSSSTQI
YQAVSRIVCGHPEGGGLKIKSLNWYEDNNYKALFGGGNGTEEDAETFYDNSTTPYCNDLMK
NLESSPLSRIIWALKAKPLLVGKILYTPDTPATRQVMAEVNKTTFQELAVFHDLEGMEELS
PKIWTFMENSQEMDLVRMLLDSRNDHFWEQQLDGLDWTAQDIVAFLAKHPEDVQSSNGS
VYTWRERAFNETNQAIRTIISRFMECVNLNKEPIATEVWLINKSMELLDERKFWAGIVFTG
ITPGSIELPHVVKYKIRMDIDNVERTNKIKDGYWDPGPRADPFEDMRYVWGGFAYLQDVV
EQAIIRVLTGTEKKTGVMQQMPYPCYVDDIFLRVMSRSMPLFMTLAWIYSVAVIIKGIV
YEKEARLKETMRIMGLDNSILWFWSFISSLIPLLVSAGLTVILKLGNNLPYSDPSVVFV
FLSVFAVVTILOCFLISTLFSRANLAAACGGIIFYFTLYLPVLCVAWQDYVGFTLKIFAS
LLSPVAFGFGCEYFALFEEQGIGVQWDNLFESPVEEDGFNLTTSVSMMLFDTFLYGVMTW
YIEAVFPQYQYGIIPRPWYFPCTKSYWFGEESDEKSHPGSNQKRISEICMEEEPTHLKLGV
IQNLVKVYRDGMKVAVDGLALNFYEGQITSFLGHNGAGKTTMSILTGLFPPTSGTAYIL
GKDIRESEMSTIRQNLGVCPQHNVLFDMLTVEEHIWFYARLKGLSEKHVKAEMEQMALDVG
LPSKSKSCKTSQLSGGMQRKLSVALAFVGGSKVVIIDEPAGVDPYSRRGIWELLKYRQ
GRTIILSTHMDEADVLDGRIAIIHGKLCCVGSSLFLKNQLGTCYYLTLVKKDVESSLS
SCRNSSSTVSYLKKEDSVSQSSSDAGLSDHESDTLTIDVSAISNLIRKHVSEARLVEDI
GHELTYVLPYEAAKEGAFVELFHIEDDRSLDLGISSYGISETTLEEFLKVAEESGVDAE
TSDGTLPARRNRRAFGDQSCLRPTEDDAADPNDSIDPESRETDLLSGMDGKGSYQVK
GWKLTQQQFVALLWKRLLIARRSRKGFFAQIVLPAVFVCIALVFSLIVPPFGKYPSELQ
PWMYNEQYTFVSNDAPEDTGTLELLNALTQDPFGTRCMEGNPIDTPCQAGEEEWTTAP
VPQTIMDLFQNGNWTMQNFSACQCSSDKIKKMLPVCPGAGGLPPPQRQNTADILQDL
TGRNISDYLVKTYVQIAKSLKNKIWVNEFRYGGFSLGVSNTOALPPSQEVNDAIKQMKK
HLKLAKDSSADRFLNSLGRFMTGLDTKNNVKWFNNKGWHAISSFLNVINNAILRANLQK
GENPSPHYGITAFNHPLNLTQQLSEVALMTTSVDVLVSICVIFAMSFVPASFVVFLIQER
VSKAKHLQFISGVKPVIIWLSNFVWDMCNYVVPATLVIIIFICFQQKSYVSSTNLPVLAL
LLLLYGSITPLMYPASFVFKIPSTAYVVLTSVNLFIGINGSVATFVLELFTDNKLNIN
DILKSVFLIFPHFCLGRGLIDMVKNQAMADALERFGENRFVSPSLSWDLVGRNLFAMAVEG
VVFFLITVLIQYRFFIRPRPVNAKLSPLNDEDVRERQRILDDGGQNDILEIKELETKI
YRRRKPAVDRICVGIPPGEFCFGLLGVNGAGKSSTFKMLTGDTVTRGDAFLNKNISLSN
IHEVHQNMGYCPQFDAITELLTGREHVEFFALLRGVPEKEVGKVGEWAIRKLGLVKYGEK
YAGNYSGGNKRKLSTAMALIGGPVFLDEPTTGMDPKARRFLWNCALSVKEGRSVVLT
SHSMEECEALCTRMAIMVNGRFRCLGSVQHLKNRGDGTYIVVRIAGSNPDLKPVQDFFG
LAFPGSVLKEHRNMLQYQLPSSLSSLARIFSILSQSKKRLHIEDYSVSQTTLDQVFVN
AKDQSDDDHLKDLSLHKNQTVVVDVAVLTSFLQDEKVKESYV

FIG. 1

(SEQ ID NO: 2):

ATGGCTTGGCCTCAGCTGAGGTGCTGCTGGAAGAACCTCACTTCAGAAGAAGACAAAC
ATGTCAGCTGCTGCTGGAAGTGGCCTGGCCTCTATTATCTCCTGATCTCTGTTCGGC
TGAGCTACCCACCCTATGAACAACATGAATGCCATTTCAAATAAGCCATGCCCTCTGCAGGA
ACACTTCCCTGGGTTCAGGGATTATCTGTAATGCCAACAAACCCCTGTTCCGTTACCCGACTCC
TGGGGAGGCTCCCGGAGTGTGGAAACCTTAACAAATCCATTGTGGCTCGCCTGTTCTCAGATG
CTCGGAGGCTCTTTATACAGCCAGAAAGACACCAGCATGAAGGCATGCGCAAAGTCTGAGA
ACATTACAGCAGATCAAGAAATCCAGCTCAAACCTGAGCTCAAGATTTCTGGTGGACAATGA
AACCTTCTCTGGGTTCTGTATCACAACCTCTCTCCCCAAAGTCTACTGTGGACAAGATGCTGA
GGGCTGATGTCAATTCTCCACAAGGTATTTGCAAGGCTACCGATTACATTGACAAGTCTGTGC
AATGGATCAAATCAGAAGAGATGATTCAACTGGTGACCAAGAAGTTCTGAGCTTGTGGCCT
ACCAAGGGAGAAACTGGCTGCAGCAGCGAGTACTTCGTTCCAACATGGACATCCTGAAGCCAA
TCCTGAGAACACTAAACTCTACATCTCCCTCCGAGCAAGGAGCTGGCTGAAGCCACAAAACA
TTGCTGCATAGTCTGGGACTCTGGCCCGAGGAGCTGTTCAAGCATGAGAAGCTGGAGTGACATGCG
ACAGGGAGGTGATGTTCTGACCAATGTGAACAGCTCCAGCTCCACCCAAATCTACCAAGGCTG
TGTCTCGTATTGTCTGCAGGCAATCCCGAGGGAGGGGGGCTGAAGATCAAGTCTCTCAACTGGTAT
GAGGACAACAACACTACAAAGCCCTTTGGAGGCAATGGACTGAGGAAGATGCTGAAGCCACAAAACA
TGACAACCTACAACCTTACTGCAATGATTGATGAAGAATTGGAGTCTAGCCTCTTCCC
GCATTATCTGAAAGCTCTGAAGCCGCTGCTGTTGGAAAGATCCTGTATACACCTGACACTCCA
GCCACAAGGCAGGTATGGCTGAGGTGAACAAGACCTTCAGGAACCTGGCTGTGTTCCATGATCT
GGAAGGCATGTGGAGGAACCTCAGCCCCAAGATCTGGACCTTCATGGAGAACAGCCAAGAAATGG
ACCTTGTCCGGATGCTGTGGACAGCAGGGACAATGACCACCTTGGAAACAGCAGTTGGATGGC
TTAGATTGGACAGCCAAGACATCGTGGCTTTGGCAAGCACCCAGAGGATGTCCAGTCCAG
TAATGGTTCTGTACACCTGGAGAGAAGCTTCAACGAGACTAACCAAGGCAATCCGGACCATAT
CTCGCTTCATGGAGTGTGTCACCTGAACAAGCTAGAACCCATAGCAACAGAACAGTCTGGCTCATC
AACAAAGTCCATGGAGCTGGATGAGAGGAAGTTCTGGGCTGGTATTGTTCACTGGAATTAC
TCCAGGCAGCATTGAGCTGCCCATCATGTCAAGTACAAGATCCGAATGGACATTGACAATGTGG
AGAGGACAATAAAATCAAGGATGGTACTGGACCCCTGGCTCGAGCTGACCCCTTGAGGAC
ATGCGGTACGCTGGGGGGCTTCGCCACTTGCAGGATGTGGCTGGAGCAGGCAATCATCAGGGT
GCTGACGGGACCGAGAAAGAAAATGGTGTCTATGCAACAGATGCCCTATCCTGTTACGTTG
ATGACATCTTCTGCGGGTGAATGAGCCGGTCAATGCCCTTCTCATGACGCTGGCTGGATTAC
TCAGTGGCTGTGATCATCAAGGGCATCGTGTATGAGAAGGAGGCACGGCTGAAAGAGACCATGCG
GATCATGGGCCATGGACAACAGCATCCTCTGGTTAGCTGGTTCAATTAGTAGCCTCATCCTCTTC
TTGAGCGCTGGCCTGCTAGTGGTCACTCTGAAGTTAGGAAACCTGCTGCCCTACAGTGTACCCC
AGCGTGGTGTGTTGCTTCTGTCCGTGTTGCTGTGGTGAACAATCCTGCAGTGTGCTTCTGATTAG
CACACTCTCTCCAGAGCCAACCTGGCAGCAGCAGCCTGTGGGGCATCATCTACTTCACGCTGTACC
TGCCTACGTCTGTGTGGCATGCCAGGACTACGTGGCTTCACACTCAAGATCTCGCTAGC
CTGCTGTCTCTGTGGCTTTGGGTTTGCTGTGAGTACTTGCCTTTGAGGAGCAGGGCAT
TGGAGTGCAGTGGACAACCTGTTGAGAGTCTGTGGAGGAAGATGGCTCAATCTCACCACCTP

FIG. 2

CGGTCTCCATGATGCTGTTGACACCTTCCTCATGGGGTATGACCTGGTACATTGAGGCTGTC
TTTCCAGGCCAGTACGGATTCCCAGGCCCTGGTATTTCTGCACCAAGTCTACTGGTTGG
CGAGGAAAGTGTGAGAAGAGCCACCCCTGGTCCAACCAGAAGAGAAATATCAGAAATCTGCATGG
AGGAGGAACCCACCCACTTGAAGCTGGCGTGTCCATTAGAACCTGGTAAAAGTCTACCGAGAT
GGGATGAAGGTGGCTGTGATGGCCTGGCACTGAATTATGAGGGCCAGATCACCTCCCT
GGGCCACAATGGAGCGGGGAAGACGACCACCATGTCATCCTGACCGGGTTGTTCCCCCGACCT
CGGGCACCGCCATACATCCTGGAAAAGACATTGCTCTGAGATGAGCACCATCCGGCAGAACCTG
GGGGTCTGTCCCCAGCATAACGTGCTGTTGACATGCTGACTGTCGAAGAACACATCTGGTTCTA
TGCCCCCTGAAAGGGCTCTGTGAGAACGACGTGAAGGGGGAGATGGAGCAGATGGCCCTGGATG
TTGGTTGCCATCAAGCAAGCTGAAAAGCAAACAGCCAGCTGTCAGGTGAATGCAAGAGAAAG
CTATCTGTGCCCTGGCCTTGTGGGGATCTAAGGTGTCAATTCTGGATGAACCCACAGCTGG
TGTGGACCCCTACTCCCCGAGGGAAATATGGGAGCTGCTGTCAGAACATACCGACAAGGCCGCACCA
TTATTCTCTACACACCACATGGATGAAGCGGACGTCTGGGGACAGGATTGCCATCATCTCC
CATGGGAAGCTGTGCTGTTGCTGGCTCCTCCCTGTTCTGAAGAACAGCTGGGAACAGGCTACTA
CCTGACCTGGTCAAGAAAGATGTGGAATCCTCCCTCAGTTCTGCAGAACAGTAGTACACTG
TGTACACCTGAAAAGGAGGACAGTGTGTTCTCAGAGCAGTTCTGATGCTGCTGGCTGGCAGCGAC
CATGAGAGTGAACGCTGACCATCGATGCTCTGCTATCTCAACCTCATCAGGAAGCATGTGTC
TGAAGCCGGCTGGTGGAAAGACATAGGGCATGAGCTGACCTATGTGCTGCCATATGAAGCTGTA
AGGAGGGAGCCTTGTGAACTCTTCATGAGATTGATGACCGGCTCTCAGACCTGGCATTCT
AGTTATGGCATCTCAGAGACGACCTGGAAGAAATATTCTCAAGGTGGCGAAGAGAGTGGGGT
GGATGCTGAGACCTCAGATGGTACCTTGCCAGCAAGACGAAACAGCGGGCTTCGGGGACAAGC
AGAGCTGCTTCGCCGTTCACTGAAGATGATGCTGCTGATCCAATGATTCTGACATAGACCCA
GAATCCAGAGAGACAGACTTGCTCAGTGGGATGGATGGCAAAGGGCTTACCAAGGTGAAAGGCTG
GAAACTTACACAGCAACAGTTGTGGCCCTTTGTGGAAGAGACTGCTAATTGCCAGACGGAGTC
GGAAAGGATTGGCTCAGATTGCTTGCAGCTGTGTTGCTGATTGCCCTTGTTGTCAG
CTGATCGTGCACCCCTTGGCAAGTACCCCAGCCTGGAACCTCAGCCCTGGATGTACAACGAACA
GTACACATTGTCAGCAATGATGCTCCTGAGGACACGGAACCCCTGGAACCTTAAACGCCCTCA
CCAAAGACCCCTGGCTTCGGGACCCGCTGTATGGAAGGAAACCCAATCCCAGACACGCCCTGCCAG
GCAGGGAGGAAGAGTGGACCACTGCCCCAGTTCCCAGACCATCATGGACCTTCCAGAATGG
GAACCTGGACAATGCAAGAACCCCTTCACCTGCATGCCAGTGTAGCAGCGACAAATCAAGAAGATGC
TGCCTGTGTCCCCCAGGGCAGGGGGCTGCCCTCCACAAAGAAAACAAACACTGCAGAT
ATCCTTCAGGACCTGACAGGAAGAAACATTGCGATTATCTGGTGAAGACGTATGTGCAAGATCAT
AGCCAAAGCTAAAGAACAAAGATCTGGGTGAATGAGTTAGGTATGGCGGCTTCCCTGGGTG
TCAGTAATACTCAAGCACTTCCCTGGAGTCAAGAAGTTAATGATGCCATCAAACAAATGAAGAAA
CACCTAAAGCTGCCAAGGACAGTTCTGCAGATCGATTCTCAACAGCTGGGAAGATTATGAC
AGGACTGGACACCAAAATAATGTCAAGGTGTGGTTCAATAACAAGGGCTGGCATGCAATCAGCT
CTTCTGAAATGTCATCAACAATGCCATTCTCCGGCCAACCTGCAAAAGGGAGAGAACCCCTAGC
CATTATGGAATTACTGCTTCAATCATCCCCCTGAATCTCACCAAGCAGCAGCTCAGAGGTGGC
TCTGATGACCACATCAGTGGATGTCCTGTGTCATCTGTGTCATCTTGCAATGTCTCGTCC
CAGCCAGCTTGTGATCCAGGAGCGGGTCAGCAAAGCAAACACCTGCAAGTTCATC
AGTGGAGTGAAGCCTGTCACTGGCTCTAATTGTCTGGGATATGTGCAATTACGTTGT

FIG. 2 (cont.)

CCCTGCCACACTGGTCATTATCATCTTCATCTGCTTCCAGCAGAAGTCCTATGTGTCCCTCACCA
ATCTGCCTGTGCTAGCCTTCTACTTTGCTGTATGGGTGGTCAATCACACCTCTCATGTACCCA
GCCTCCTTGTGTTCAAGATCCCCAGCACAGCCTATGTGGTGCTCACCAGCGTGAACCTCTCAT
TGGCATTAAATGGCAGCGTGGCCACCTTGTGCTGGAGCTGTTACCGACAATAAGCTGAATAATA
TCAATGATATCCTGAAGTCCGTGTTGATCTCCCACATTTTGCCCTGGGACGAGGGCTCATC
GACATGGTCAAAAACCAGGCAATGGCTGATGCCCTGGAAAGGTTGGGAGAATCGCTTGTGTC
ACCATTATCTGGGACTTGGTGGGACGAAACCTTCCGCATGGCGTGGAAAGGGGTGGTGTCT
TCCTCATTACTGTTCTGATCCAGTACAGATTCTCATCAGGCCAGACCTGTAATGCAAAGCTA
TCTCCTCTGAATGATGAAGATGAAGATGTGAGGGCGGAAAGACAGAGAATTCTTGATGGTGGAGG
CCAGAACATGACATCTAGAAATCAAGGAGTTGACGAAGATAATAGAAGGAAGCGGAAGCCTGCTG
TTGACAGGATTGCGTGGGCATTCCCTGTTGAGTGCTTGGGCTCTGGAGTTAATGGGCT
GGAAAATCATCAACTTCAAGATGTTAACAGGAGATACCAACTGTTACCAAGAGGAGATGCTTTCCT
TAACAAAAATAGTATCTTATCAAACATCCATGAAGTACATCAGAACATGGCTACTGCCCTCACT
TTGATGCCATCACAGAGCTGTTGACTGGGAGAGAACACGTGGAGTTCTTGCCCTTTGAGAGGA
GTCCCAGAGAAAGTTGGCAAGGTTGGTGGAGTGGGCGATTGGAAACTGGGCTCGTGAAGTA
TGGAGAAAAATATGCTGGTAACTATAGTGGGAGCAACAAACCCAAAGCTCTACAGCCATGGCTT
TGATCGGGGGGCTCTGTGGTGTGTTCTGGATGAACCCACACAGGCATGGATCCAAAGCCGG
CGGTTCTGTGAAATTGTCCTAAGTGTCAAGGAGGGAGATCAGTAGTGTCTACATCTCA
TAGTATGGAAGAATGTGAAGCTTTGCACTAGGATGGCAATCATGGTCAATGGAAGGTTAGGT
GCCTTGGCAGTGTCCAGCATCTAAAAAAATAGGTTGGAGATGGTTATAAATAGTTGTACGAATA
GCAGGGTCCAACCCGACCTGAAGCCTGTCAGGATTCTTGACTTGCAATTCTGCTTGGAAAGTGT
TCTAAAAGAGAAACACCGAACATGCTACAATACCAAGCTTCCATCTCATTATCTCTGCGCCA
GGATATTCAAGCATCCTCTCCCAAGAGCAAAAGCGACTCCACATAGAAGACTACTCTGTTCTCAG
ACAACACTGACCAAGTATTGTGAACITGGCAAGGACCAAGTGAATGACCACTTAAAGA
CCTCTCATTACACAAAAACAGACAGTAGTAGTGGACGTTGCAGTTCTCACATCTTACAGGATG
AGAAAGTGAAGAAAGCTATGTA

FIG. 2 (cont.)

(SEQ ID NO: 3):

AACAAAATATTAACGCTTACAATTCCATTGCCATTAGGCTGCCAACTGTGGGAAGGGCGA
TCGGTGCAGGGCCTCTCGCTATTACGCCAGCTGGCGAAAGGGGATGTGCTGCAAGGCATAAG
TTGGGTAACGCCAGGGTTTCCCAGTCACGACGGTGTAAAACGACGGCCAGTCCAAGCTGATCT
ATACATTGAATCAATATTGGCAATTAGCCATTAGTCATTGGTTATATAGCATAAATCAATATT
GGCTATTGGCCATTGCATACGTTGTATCTATATCATAAATATGTACATTATATTGGCTCATGTCC
AATATGACGCCATGTTGACATTGATTATGACTAGTTATAAGTAATCAATTACGGGTCAT
TAGTTCATAGCCCCATATATGGAGTTCGCCGTTACATAACTACGGTAAATGGCCCGCTGGCTGA
CCGCCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGG
GACTTTCCATTGACGTCAATGGGGAGTATTACGGTAAACTGCCACTTGGCAGTACATCAAG
TGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTAT
GCCCACTACATGACCTTACGGGACTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTAT
TACCATGGTGTGCGGTTTGGCAGTACACCAATGGGCGTGGATAGCGGTTGACTCACGGGAT
TTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTGGTTGGCACAAAATCAACGGGACTTT
CCAAAATGTCGTAATAACCCCGCCCCGTTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGT
CTATATAAGCAGAGCTCGTTAGTGAAACCGTCAGAATTGTAATACGACTCATAGGGCGC
CGGGAAATCGTCGACTGGATCCGGTACCGAGGGAGATCTGCCGCCGATGCCATGGCTTGG
CCTCAGCTGAGGTTGCTGCTGTTGGAAGAACCTCAGTTTCAAAGAACATGTCAGCTGCT
GCTGGAAGTGGCCTGCCCTATTTATCTTCTGATCTGATCTCTGTTGGCTGAGCTACCCAC
CCTATGAACAACATGAATGCCATTTCAAATAAGCCATGCCCTCTGCAAGAACACTTCTTGG
GTTCAAGGGATTATCTGTAATGCCAACAACCCCTGTTCCGTTACCGACTCCTGGGGAGGCTCC
CGGAGTTGAAACTTAAACAAATCCATTGCGCTGCCCTGTTCTCAGATGCTCGGAGGCTTC
TTTATACAGCCAGAAAAGACACCAGCATGAAGGACATGCCAAAGTTCTGAGAACATTACAGCAG
ATCAAGAAATCCAGCTCAAACCTGAAGCTCAAGATTCTGGTGGACAATGAAACCTCTCTGG
GTTCCCTGATCACAAACCTCTCTCCAAAGTCTACTGTGGACAAGATGCTGAGGGCTGATGTCA
TTCTCCACAAGGTATTTTGCAAGGCTACCAAGTTACATTGACAAGTCTGTGCAATGGATCAAAA
TCAGAAAGAGATGATCAACTTGGTGAACAGAAGAAGTTCTGAGCTTGTGGCTACCAAGGGAGAA
ACTGGCTGCAGCAGAGCAGTACTTCGTTCCAACATGGACATCTGCAAGCCAATCTGAGAACAC
TAAAACCTACATCTCCCTCCGAGCAAGGAGCTGGCTGAAGGCCACAAAACATTGCTGCATAGT
CTTGGGACTCTGCCAGGAGCTGTCAGCATGAGAAGCTGGAGTGACATGCGACAGGAGGTGAT
GTTTCTGACCAATGTGAACAGCTCCAGCTCTCCACCCAAATCTACCAAGGCTGTTCTCGTATTG
TCTGCGGCATCCGAGGGAGGGGGCTGAAGATCAAGTCTCTCAACTGGTATGAGGACAACAAAC
TACAAAGCCCTTTGGAGGCAATGGCACTGAGGAAGATGCTGAACCTTCTATGACAACCTAC
AACTCCTACTGCAATGATTGATGAAGAATTGGAGTCTAGTCCCTTTCCGCATTATCTGGA
AAGCTCTGAAGCCGCTGCTGGAGAAGATCCTGTATACACCTGACACTCCAGCCACAAGGCAG
GTCATGGCTGAGGTGAACAAGACCTCCAGGAACGGCTGTGTTCCATGATCTGGAGGGATGTG
GGAGGAACCTAGCCCCAAGATCTGGACCTTCATGGAGAACAGCCAAGAAATGGACCTTGTCCGGA

FIG. 3

TGCTGTTGGACAGCAGGGACAATGACCAC TTTGGGAACAGCAGTTGGATGGCTTAGATTGGACA
GCCCAAGACATCGTGGCTTTGGCCAAGCACCCAGAGGATGTCCAGTCCAGTAATGGTTCTGT
GTACACCTGGAGAGAAGCTTCACAGAGACTAACCGAGGCAATCCGGACCATACTCGCTTCATGG
AGTGTGTCAACCTGAACAAGCTAGAACCCATAGCAACAGAAGTCTGGCTCATCAACAAGTCCATG
GAGCTGCTGGATGAGAGGAAGTTCTGGCTGGTATTGTGTCACTGGAATTACTCCAGGCAGCAT
TGAGCTGCCCATCATGTCAGTACAAGATCCGAATGGACATTGACAAATGTGGAGAGGACAATA
AAATCAAGGATGGTACTGGGACCCCTGGCTCTCGAGCTGACCCCTTGGAGGACATGCCGTACGTC
TGGGGGGCTCGCCTACTTGCAAGGATGTGGTGGAGCAGGAATCATCAGGGTGTGACGGGCAC
CGAGAAGAAAACCTGGTGTCTATATGCAACAGATGCCCTATCCCTGTTACGTTGATGACATTTTC
TGCGGGTGTGAGCCGGTCAATGCCCTCTTCATGACGCTGGCTGGATTACTCAGTGGCTGTG
ATCATCAAGGGCATCGTGTATGAGAAGGAGGCACGGCTGAAAGAGACATGCCGTACGGGATCATGGGCT
GGACAACAGCATTCTCGTGTAGCTGGTTAGCTGGTCATTAGTAGCCTCATTCTCTTCTTGTGAGCGCTG
GCCTGCTAGTGGTCAATCCTGCAAGTTAGGAAACCTGCTGCCCTACAGTGTACCCACGCTGGTGT
GTCTTCTGTCCGTGTTGCTGTGGTGTGACAATCCTGCAAGTGTACCTGCTGCTGTCT
CAGAGCCAACCTGGCAGCAGCCTGTGGGGCATCATCTACTTACGCTGTACCTGCCCTACGTC
TGTGTGTGGCATGGCAGGACTACGTGGCTTCACACTCAAGATCTTCGCTAGCCTGCTGTCT
GTGGCTTTGGGTTGGCTGTGAGTACTTGGCCCTTTGAGGAGCAGGGCATTGGAGTGCAGTG
GGACAACCTGTTGAGAGTCCTGTGGAGGAAGATGGCTCAATCTACCAACTTCGGTCTCCATGA
TGCTGTTGACACCTCCCTATGGGTGATGACCTGGTACATTGAGGCTGTCTTCCAGGCCAG
TACGGAATTCCCAGGCCCTGGTATTTCTTGACCAAGTCTACTGTTGGCAGGAAAGTGA
TGAGAAGAGCCACCCCTGGTCAACCAGAAGAGAATATCAGAAATCTGCACTGGAGGAGGAACCCA
CCCACTTGAAGCTGGCGTGTCCATTCAAGACCTGGTAAAGTCTACCGAGATGGGATGAAGGTG
GCTGTCGATGGCCTGGCAGTGAATTATGAGGGCCAGATCACCTCTTGGCACAATGG
AGCGGGGAAGACGACCACATGTCATCCTGACCGGGTTGTTCCCCCGACCTGGCACCCT
ACATCCTGGAAAAGACATTGCTCTGAGATGAGCACCACCGCAGAACCTGGGGTCTGTC
CAGCATAACGTGCTGTTGACATGCTGACTGTCGAAGAACACATCTGGTCTATGCCGCTTGA
AGGGCTCTGTGAGAAGCAGTGAAGCGGAGATGGAGCAGATGGCCCTGGATGTTGGTTGCCAT
CAAGCAAGCTGAAAAGCAAACAGCCAGCTGTCAAGGTGGATCTGGATGAAACCCACAGCTGGTGTG
TTGGCCTTGTGGGGGATCTAAGGGTGTCTGGATGAAACCCACAGCTGGTGTGACCT
CTCCCGCAGGGGAATATGGGAGCTGCTGTGAAATACCGACAAGGCCGACCATTATTCTCT
CACACCCACATGGATGAAGCGGACGTCTGGGGACAGGATTGCCATCATCTCCATGGGAAGCTG
TGCTGTTGGCTCCTCCCTGTTCTGAAGAACCCAGCTGGAAACAGGCTACTACCTGACCTGGT
CAAGAAAGATGTGAATCTCCCTCAGTTCTGCAAGAACAGTAGTACGACTGTGTCAACCTGA
AAAAGGAGGACAGTGTGTTCTCAGAGCAGTGTGATGCTGGCTGGCAGCGACCATGAGAGTGAC
ACGCTGACCATCGATGTCTGCTATCTCCACCTCATCAGGAAGCATGTGTCAAGGCCGGCT
GGTGGAAAGACATAGGGCATGAGCTGACCTATGTGCTGCCATATGAAGCTGCTAAGGAGGGAGCCT
TTGTGGAACTCTTCATGAGATTGATGACCGGGCTCTCAGACCTGGCATTCTAGTTATGGC
TCAGAGACGACCCCTGGAAAGAAATATCCTCAAGGTGGCCGAAGAGAGTGGGGTGGATGCTGAGAC
CTCAGATGGTACCTTGCAGCAAGACGAAACAGGCCGGCCTCGGGGACAAGCAGAGCTGCTTC

FIG. 3 (cont.)

GCCCCTTCACTGAAGATGATGCTGCTGATCCAAATGATTCTGACATAGACCCAGAATCCAGAGAG
ACAGACTTGCTCAGTGGGATGGATGCCAAGGGCCTACCAGGTGAAAGGCTGAAACTTACACA
GCAACAGTTGTGGCCCTTTGTGAAAGAGACTGCTAATTGCCAGACGGAGTCGAAAGGATT
TTGCTCAGATTGTCTTGCCAGCTGTGTTGCTGCATTGCCCTGTGTTGCTGAGCCTGATCGGCCA
CCCTTGGCAAGTACCCCAGCCTGAACTTCAGCCCTGGATGTCACACAGAACAGTACACATTGT
CAGCAATGATGCTCCTGAGGACACGGAAACCTGAACTCTAAACGCCCTCACCAAAGACCC
GCTTCGGGACCCGCTGTATGAAAGGAAACCCAATCCCAGACACGCCCTGCCAGGCAGGGGAGGAA
GAGTGGACCACTGCCCAAGTCCCCAGACCATCATGGACCTCTTCCAGAATGGAACTGGACAAT
GCAGAACCCCTTCACCTGCATGCCAGTGTAGCAGCGACAAAATCAAGAAGATGCTGCCCTGTGTC
CCCCAGGGGAGGGGGCTGCCCTCCACAAAGAAAACAAAACACTGCAGATATCCTTCAGGAC
CTGACAGGAAGAACATTCCGATTATCTGGTGAAGACGTATGTGCAGATCATAGCCAAAAGCTT
AAAGAACAAAGATCTGGTGAATGAGTTAGGTATGGCGGTTTCCCTGGGTGTCAGTAATACTC
AAGCACTCCCTCCGAGTCAGAAGTTAATGATGCCATCAAACAAATGAAGAACACCTAAAGCTG
GCCAAGGACAGTCTGCAGATCGATTCTCAACAGCTTGGGAGATTATGACAGGACTGGACAC
AAAAAAATAATGTCAGGGTGTGTTCAATAACAAGGGCTGCCATGCAATCAGCTTTCCGTAAATG
TCATCAACAATGCCATTCTCCGGCCAACCTGCAAAAGGGAGAGAACCTAGCCATTATGGAATT
ACTGCTTCAATCATCCCCCTGAATCTCACCAAGCAGCAGCTCTCAGAGGTGGCTCTGATGACCAC
ATCAGTGGATGTCCTGTGTCATCTTGCAATGTCCTCGTCCCAGCCAGCTTG
TCGTTCTGATCCAGGAGCGGGTCAAGCAAAACACCTGCAGTTCATCAGTGGAGTGAAG
CCTGTCATCTACTGGCTCTCAATTGTGCTGGATATGTCATTACGTTGCTCCCTGCCACACT
GGTCATTATCATCTGCTTCAGCAGAAGTCCTATGTCCTCCACCAATCTGCCGTG
TAGCCCTCTACTTTGCTGTATGGGTGGTCAATCACACCTCTCATGTACCCAGCCTCTTG
TTCAAGATCCCCAGCACAGCCTATGTTGCTCACCGCGTGAACCTCTTCATTGGCATTAAATGG
CAGCGTGGCCACCTTGTGCTGGAGCTGTCACCGACAATAAGCTGAATAATCAATGATATCC
TGAAGTCCGTGTTCTTGATCTTCCACATTGTCCTGGGACGAGGGCTCATCGACATGGTAAA
AACCAAGGCAATGGCTGATGCCCTGAAAGGTTGGGGAGAATCGCTTGTGTCACCATTATCTG
GGACTTGGTGGGAGAACCTCTGCCATGGCGTGGAGGGTGGTCTTCCCTCATTACTG
TTCTGATCCAGTACAGATTCTCATCAGGCCAGACCTGTAATGCAAAGCTATCTCTCTGAAT
GATGAAGATGAAGATGTGAGGCGGGAAAGACAGAGAATTCTGATGGTGGAGGCAGAATGACAT
CTTAGAAATCAAGGAGTTGACGAAGATATAGAAGGAAGCGGAAGCCTGCTGTTGACAGGATT
GCGTGGGCATTCCCTCTGGTGAAGTGTGTTGGCTCTGGGAGTTAATGGGCTGAAAATCATCA
ACTTCAGATGTTAACAGGAGATACCAACTGTTACCAAGAGGAGATGCTTCCCTAAACAAAATAG
TATCTTATCAAACATCCATGAACTACATCAGAACATGGCTACTGCCCTCAGTTGATGCCATCA
CAGAGCTGTTGACTGGGAGAGAACACGTTGGAGTTCTTGCCCTTGTGAGAGGAGTCCCAGAGAAA
GAAGTTGGCAAGGTTGGTGAAGTGGGAGAACCTGGGCTCGTGAAGTATGGAGAAAAATA
TGCTGGTAACTATAGTGGAGGCAACAAACGCAAGCTCTACAGCCATGGCTTGATCGGCGGGC
CTCCGTGGTGTCTGGATGAACCCACCACAGGAGTGGATCCAAAGGCCGGGTTCTGTGG
AATTGTGCCCTAAGTGTGTCAGGGAGGGAGATCAGTAGTGTGCTTACATCTCATAGTATGGAAGA
ATGTGAAGCTTTGCACTAGGATGGCAATCATGGTCAATGGAAGGTTAGGTGCCCTGGCAGTG
TCCAGCATCTAAAAAATAGGTTGGAGATGGTTACAATAGTTGTAAGCAATAGCAGGGTCCAAC

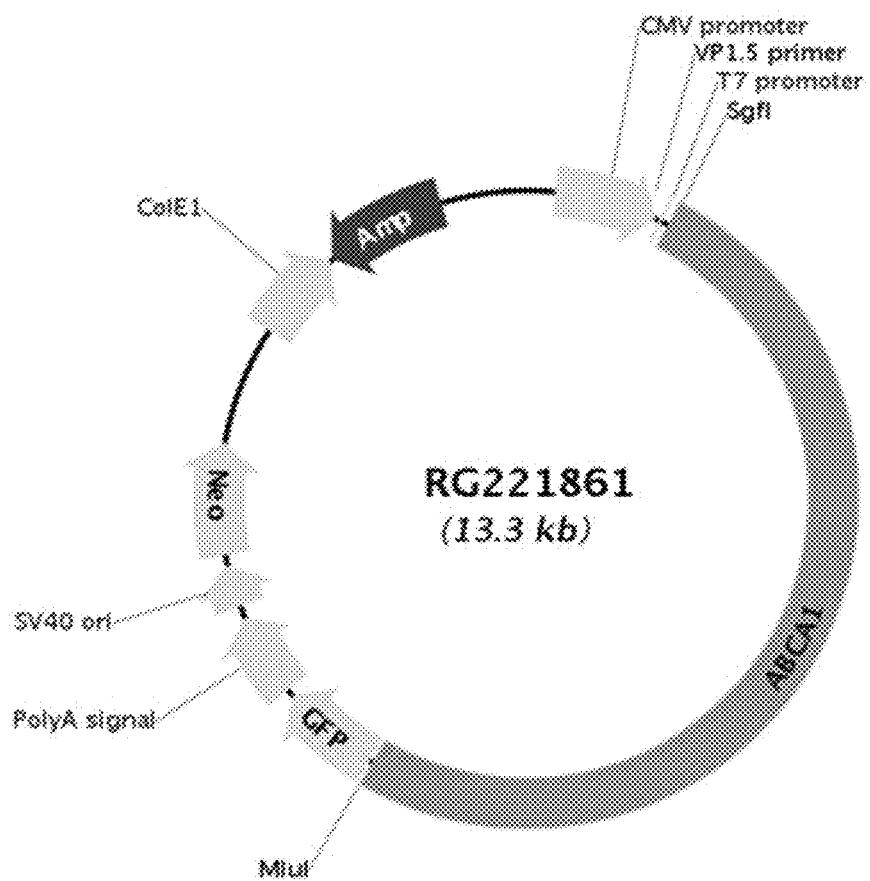
FIG. 3 (cont.)

CCGGACCTGAAGCCTGCCAGGATTCTTGACTTGCATTTCTGGAAAGTGTCTAAAAGAGAA
ACACCGGAACATGCTACAATAACCAAGCTTCCATCTTCATTATCTTCTCTGCCAGGATATTCA
GCAGTATTGTGAACCTTGCAAGGACCAAAGTGTGATGACCCTAAAGACCTCTCATTACA
CAAAAACCAGACAGTAGTGGACGTTGCAGTTCTCACATCTTCTACAGGATGAGAAAGTGAAAG
AAAGCTATGTAACCGCTACGCCGCGCTCGAGCAGAAACTCATCTCAGAAGAGGATCTGGCAGCA
AATGATATCCTGGATTACAAGGATGACGACGATAAGGTTAACGGCCGCCGGTCAAGCTG
TTTCTGAACAGATCCCCGGTGGCATCCCTGTGACCCCTCCCCAGTGCCTCTCCTGGCCCTGGAA
GTTGCCACTCCAGTGCCACCAGCCTGTCTAATAAAATTAAGTGCATCATTTGTCTGACTA
GGTGTCTTCTATAATATTATGGGGTGGAGGGGGTGGTATGGAGCAAGGGCAAGTTGGGAAGA
CAACCTGTAGGGCCTGCCGGTCTATTGGGAACCAAGCTGGAGTGCAGTGGCACAAATCTGGCTC
ACTGCAATCTCCGCCTCTGGGTTCAAGCGATTCTCCTGCCCTAGCCTCCCAGGTTGGGATT
CCAGGCATGCATGACCAGGCTCAGCTAATTTTGTGTTGGTAGAGACGGGGTTCACCATAT
TGGCCAGGCTGGTCTCCAACCTCTAATCTCAGGTGATCTACCCACCTTGGCCTCCAAATTGCTG
GGATTACAGGCGTGAACCAACTGCTCCCTTCCCTGTCTGATTAAAATAACTATACAGCA
GGAGGACGTCCAGACACAGCATAGCTACCTGGCATGCCAACCGGTGGACATTGAGTTGCT
TGCTTGGCAGTGTCTCATGCCTGGTCCACTCAGTAGATGCCTGTTGAATTGGGTACGCC
CCAGCGGCAGCGGTATCAGCTACTCAAAGCGGTAAATACGGTTATCCACAGAAATCAGGGATA
ACGCAGGAAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAGGCCCGTTG
CTGGCGTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAG
GTGGCGAAACCGACAGGACTATAAGATAACAGGGCTTCCCTGGAGCTCCCTCGTGC
CTCCCTGTTCCGACCCCTGCCGCTTACCGGATACTGTCCGCTTCTCCCTCGGAAGCGTGGCG
CTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCTCGCTCAAGCTGGCTG
TGTGCACGAACCCCCCGTTCAGCCGACCGCTGCCCTATCCGGTAACTATCGTCTTGAGTCCA
ACCCGGTAAGACACGACTATGCCACTGGCAGCCACTGGTAACAGGATTACAGAGCGAGG
TATGTAGGCGGTGCTACAGAGTTCTGAAGTGGTGGCTAACTACGGCTACACTAGAAGAACAGT
ATTGGTATCTGCCTCTGCTGAAGCCAGTTACCTCGAAAAAGAGTTGGTAGCTCTTGATCCG
GCAAACAAACCAACCGCTGGTAGCGGTGGTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAA
AAAGGATCTCAAGAACATCCTTGATCTTCTACGGGTCTGACGCTCAGTGGAACGAAACCTC
ACGTTAAGGATTGGTCACTGAGATTATCAAAAGGATCTCACCTAGATCTTAAATTAAA
AATGAAGTTTAAATCAATCTAAAGTATATGAGTAACCTGAGGCTATGGCAGGGCCTGCC
CCGACGTTGGCTGCCAGGCCCTGGGCTTCACCGAACTTGGGGGGTGGGGGGAAAAGGAAGA
AACCGGGCGTATTGGCCCCAATGGGGTCTCGGTGGGTATCGACAGAGTGCACGCCCTGGGACC
GAACCCCGCGTTATGAACAAACGACCAACACCGTGCCTTATTCTGTCTTTATTGCCGTC
ATAGCGCGGGTCTCCCGTATTGTCTCTCCGTGTTCAAGTACGCTCCCTAGGGTGGC
GAAGAACTCCAGCATGAGATCCCCCGCTGGAGGATCATCCAGCCGGTCCCGAAAACGATTC
CGAAGCCCAACCTTCATAGAAGGCGCGTGGATACCGTAAAGCACGAGGAAGCGGTCA
GCTGGTCGGTCATTCTGAACCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGC
GCGATGCGCTCGAATCGGGAGCGCGATACCGTAAAGCACGAGGAAGCGGTCA
GCCAAGCTCTCAGCAATATCACGGTAGCCAACGCTATGTCTGATAGCGATCCGCC
GCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTCACCATGATATTGGCAAGCAGGCA

FIG. 3 (cont.)

TCGCCATGGGTACGACGAGATCCTCGCCGTGGCATGCTGCCCTTGAGCCTGGCAACAGTTC
GGCTGGCGCGAGCCCTGATGCTCTCGTCCAGATCATCCTGATCGACAAGACGGCTTCCATCC
GAGTACGTGCTCGCTCGATGCGATGTTCGCTGGTGGTGAATGGCAGGTAGCCGGATCAAGC
GTATGCAGCCGCCGATTGCATCAGCCATGATGGATACTTCTCGCAGGAGCAAGGTGAGATGA
CAGGAGATCCTGCCCGCACTTCGCCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGT
CGAGCACAGCTGCGCAAGGAACGCCGTGCGGCCAGCCACGATAGCCGCGTGCCTCGTCTGC
AGTCATTCAAGGCACCGCACAGGTCGGTCTTGACAAAAAGAACGGGCGCCCTGCGCTGACAG
CCGGAACACGGCGGCATCAGAGCAGCCATTGTCGTGTCAGTCATAGCCGAATAGCCTCT
CCACCCAAGCGGCCGGAGAACCTGCGTGCATTCATCTGTTCAATCATGCGAAACGATCCTCAT
CCTGTCCTTGATCGATCTTGCAAAAGCCTAGGCCTCCAAAAAAGCCTCTCACTACTCTGG
ATAGCTCAGAGGCCGAGGCCCTCGGCCTCTGCATAAATAAAAAAATTAGTCAGCCATGGGG
GGAGAATGGCGGAACGGGGAGTTAGGGCGGGATGGGGGGAGTAGGGGGGGACTATGGT
TGCTGACTAATTGAGATGCATGCTTGATCATACTCTGCCTGCTGGGGAGCCTGGGGACTTTCCAC
ACCTGGTTGCTGACTAATTGAGATGCATGCTTGATCATACTCTGCCTGCTGGGGAGCCTGGGGAC
TTTCCACACCTTAACGTGACACACATCCACAGCTGGTCTTCCGCCTCAGGACTCTCCTTTT
CAATATTATTGAAGCATTATCAGGGTTATTGTCATGAGCGGATACATATTGAATGTATT
AAAAAATAACAAATAGGGTTCCCGCAGACATTCCCCGAAAAGTGCACCTGACGCCCTGTA
GCGCGCATTAGCGCGGGGTGGTGGTTACGCGCAGCGTACAGCTGCCAGCGCC
CTAGCGCCCGCTCTTCTGCTTCTCCCTTCTGCCACGTTGCCGGCTTCCCCCTCA
AGCTCTAAATGGGGCTCCCTTAGGGTCCGATTAGTGCCTTACGGCACCTCGACCCAAAA
AACTTGATTAGGGTGTGTTACGTTAGTGGACTTTGTTCAAACGGTTTTGCCCTTTG
ACGTTGGAGTCCACGTTCTTAATAGTGGACTTTGTTCAAACGGTAAACACTCAACCCTAT
CTCGGTCTATTCTTTGATTATAAGGGATTGCGGATTGCGCTATTGGTAAAAATGAGC
TGATTAAACAAAATTAAACGCAATTAAACAAAATT

FIG. 3 (cont.)

**FIG. 4**

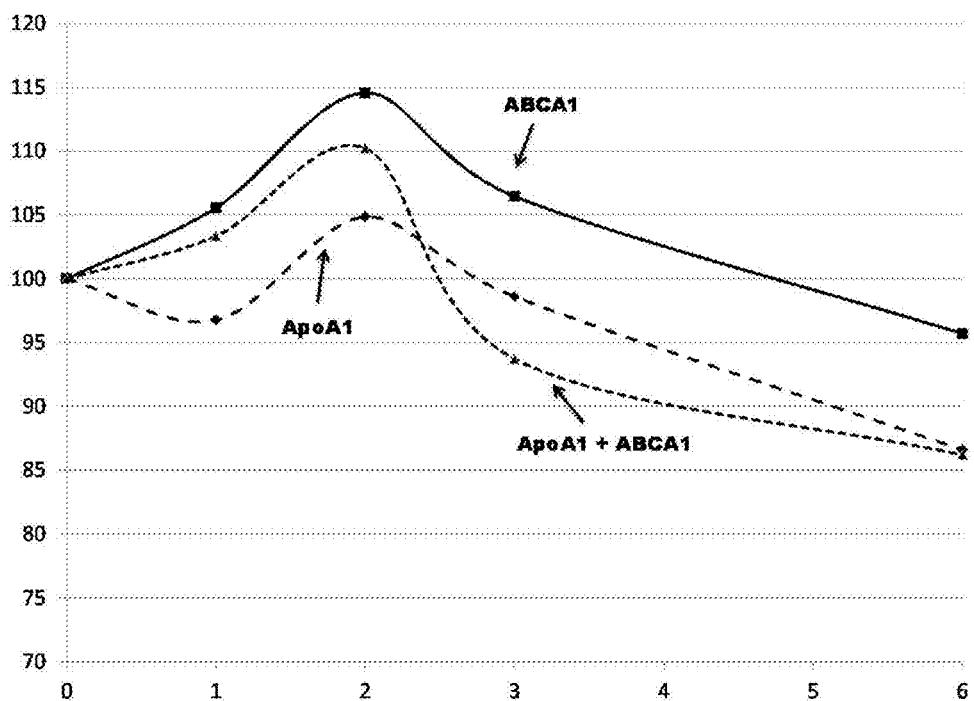


FIG. 5

1

**SONOCHEMICAL INDUCTION OF ABCA1
EXPRESSION AND COMPOSITIONS
THEREFOR**

FIELD OF THE INVENTION

This invention relates to compositions comprising sonochemically-active microspheres and a plasmid encoding ATP-binding cassette transporter A1, and methods of inducing ATP-binding cassette transporter A1 expression in vivo.

SEQUENCE LISTING INCORPORATION

Biological sequence information for this application is included in an ASCII text file having the file name "SG-2-SEQ-4_ST25.txt", created on Sep. 10, 2013 and having a file size of 44617 bytes, which is incorporated herein by reference.

BACKGROUND

High-density lipoprotein (HDL) is the largest of the five major groups of lipoproteins. Other lipoproteins include chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and low-density lipoprotein (LDL). HDL and other lipoproteins enable cholesterol and other lipids (e.g., triglycerides) to be transported within the bloodstream, despite their hydrophobic nature. About thirty percent of blood cholesterol is carried by HDL in healthy adults.

ATP-binding cassette transporter A1 (ABCA1) is an integral cell membrane protein that exports excess cholesterol from cells in conjunction with phospholipid that is necessary for the initial lipidation of ApoA1 to form nascent high density lipoprotein cholesterol (HDL-c). ABCA1 deficiency leads to very low plasma levels of HDL-c. In contrast, ABCA1 overexpression reportedly protected C57B1/6 mice from diet-induced atherosclerosis. Increasing ABCA1 transcription by enhancing its regulatory gene control by liver X factor (LXR) has led to the induction of not only HDL biogenesis, but also to an adverse increase in lipogenesis, leading to undesirable hepatic steatosis.

HDL-c is formed in the liver and the intestines by the lipidation of apolipoprotein A1 (apoA1) mediated by the ABCA1 transporter protein. Numerous studies of cultured cells, human HDL deficiencies, and animal models have shown that ABCA1 is a major determinant of plasma HDL levels and a potent atheroprotective factor. The role of ABCA1 in the liver for the formation of plasma HDL is well established, but it has been unclear whether stimulation of ABCA1 production will enhance lipidation and secretion of nascent HDL thereby resulting in an increase in plasma HDL-c levels.

The metabolism of HDL is complex and several factors contribute to the transport of cholesterol from arteries to the liver for excretion or re-use (reverse cholesterol transport). Some key players in reverse cholesterol transport include ABCA1, ABCG1, apoA1, apoE, LXR, Niemann Pick proteins 1 and 2 (NPC 1 and NPC2) sterol regulating element binding protein (SREBP), CD36, acyl cholesterol acyl transferase (ACAT) and scavenger receptor A1 (SRA1). Many of these proteins have been considered as drug targets for enhancing blood HDL-c levels; however, the effects of targeting, enhancing the activity of, or otherwise interfering with the normal activity and expression of any of these individual proteins is unpredictable. High cholesterol also commonly is treated by inhibiting production of cholesterol in the

2

liver (e.g., by inhibiting HMG-CoA reductase) or by inhibiting digestion of fats (e.g., by inhibiting bile acid production).

Plasma HDL-c and LDL-c levels are routinely measured as indicators of systemic atherosclerosis resulting in arterial blockage. HDL can remove cholesterol from within arteries and transport the cholesterol back to the liver for excretion or re-utilization. Individuals with higher levels of HDL-c have a reduced tendency for cardiovascular diseases. Low HDL-c cholesterol levels (less than about 40 mg/dL or about 1 mmol/L) are associated with increased risk of heart disease. In patients with Tangier disease (also known as "familial alpha-lipoprotein deficiency"), a rare inherited disorder, mutations in chromosome 9q31 lead to an inactive form of ABCA1. The inactive ABCA1 leads to severely depressed levels of HDL in the blood. Currently, there is no effective treatment for Tangier disease.

Because of the positive epidemiological correlation between HDL-c levels in the blood and reduced risk of heart disease, as well as the link between HDL and Tangier disease, there is an ongoing need for new methods of increasing blood HDL-c levels. The present application addresses this ongoing need.

SUMMARY OF THE INVENTION

The present invention provides compositions useful for transfecting cells (e.g., liver cells) to express ABCA1. The compositions described herein comprise a pharmaceutically acceptable aqueous carrier containing sonochemically-active microspheres together with a plasmid DNA construct encoding an active form of ABCA1 and at least one promoter for the expression thereof. The sonochemically-active microspheres comprise, consist essentially of, or consist of gas bubbles (e.g., a fluorocarbon gas, such as octafluoropropane or perfluorohexane) encapsulated within protein-containing or lipid-containing shells (e.g., human serum albumin shells). The microspheres are disruptable by exposure to ultrasonic energy (sonication) to release the encapsulated gas.

The following embodiments are provided as illustrative, non-limiting examples of the compositions and methods described herein.

Embodiment 1 comprises a composition useful for transfecting cells comprising a mixture of a plasmid vector encoding an active form of ATP-binding cassette transporter A1 (ABCA1) and sonochemically-active microspheres in a pharmaceutically acceptable aqueous carrier. The vector comprises an expressible open reading frame encoding the active form of ABCA1 and at least one sequence adapted to promote expression of the open reading frame in a mammalian cell. The sonochemically-active microspheres comprise gas bubbles encapsulated within shells comprising a protein, a lipid, or a combination thereof, the microspheres being disruptable upon exposure to ultrasonic acoustic energy to release the encapsulated gas bubbles.

Embodiment 2 comprises the composition of embodiment 1 wherein the microspheres have an average particle size in the range of about 0.5 to about 20 micrometers.

Embodiment 3 comprises the composition of embodiment 1 or embodiment 2 wherein the gas comprises a fluorocarbon gas.

Embodiment 4 comprises the composition of any one of embodiments 1 to 3 wherein the shells comprise human serum albumin.

Embodiment 5 comprises the composition of any one of embodiments 1 to 4 wherein the active form of ABCA1 has the amino acid sequence of SEQ ID NO: 1.

Embodiment 6 comprises the composition of any one of embodiments 1 to 5 wherein the open reading frame has the nucleotide sequence of SEQ ID NO: 2.

Embodiment 7 comprises the composition of any one of embodiments 1 to 6 wherein the at least one sequence adapted to promote expression of the open reading frame comprises a cytomegalovirus promoter.

Embodiment 8 comprises the composition of any one of embodiments 1 to 7 wherein the plasmid is present in the composition at a concentration in the range of about 0.5 to about 50 mg/mL.

Embodiment 9 comprises the composition of any one of embodiments 1 to 8 wherein the microspheres are present in the composition at a concentration in the range of about 10^8 to about 10^9 microspheres per milliliter.

Embodiment 10 comprises the composition of any one of embodiments 1 to 9 wherein the aqueous carrier comprises physiological saline, optionally buffered at physiological pH.

Embodiment 11 comprises the composition of any one of embodiments 1 to 10 further comprising at least one material selected from the group consisting of (a) a drug for treating a condition relating to lipid metabolism or transport, (b) a plasmid encoding a protein other than ABCA1 involved in lipid metabolism of transport, and (c) a plasmid encoding an siRNA that targets a protein involved in lipid metabolism or transport.

Embodiment 12 comprises the composition of any one of embodiments 1 to 11 wherein the plasmid encoding ABCA1 also encodes at least one material selected from the group consisting of (a) a protein other than ABCA1 involved in lipid metabolism of transport, and (b) an siRNA that targets a protein involved in lipid metabolism or transport.

Embodiment 13 comprises a composition useful for transfecting cells comprising a mixture of about 0.5 to about 50 mg/mL of a plasmid vector encoding an active form of ATP-binding cassette transporter A1 (ABCA1) and about 10^8 to about 10^9 microspheres per milliliter of sonochemically-active microspheres in a pharmaceutically acceptable aqueous carrier; wherein the vector comprises an expressible open reading frame encoding the active form of ABCA1 and at least one sequence adapted to promote expression of the open reading frame in a mammalian cell; and wherein the sonochemically-active microspheres comprise octafluoropropane gas bubbles encapsulated within shells comprising human serum albumin, the microspheres being disruptable upon exposure to ultrasonic acoustic energy to release the encapsulated gas bubbles.

Embodiment 14 comprises a method of transfecting a tissue *in vivo* to express an active form of ABCA1 in cells of the tissue, the method comprising the steps of (a) intravenously co-administering to a subject a plasmid vector encoding the active form of ATP-binding cassette transporter A1 (ABCA1), and sonochemically-active microspheres; wherein the vector comprises an expressible open reading frame encoding the active form of ABCA1 and at least one sequence adapted to promote expression of the open reading frame in a mammalian cell; and wherein the sonochemically-active microspheres comprise gas bubbles encapsulated within shells comprising a protein, a lipid, or a combination thereof, the microspheres being disruptable upon exposure to ultrasonic acoustic energy to release the encapsulated gas bubbles; (b) ultrasonically imaging the tissue of the subject to be transfected while the plasmid and microspheres of the composition are circulating through the vasculature of the tissue and thereby detecting the presence of the microspheres in the vasculature of the tissue; and (c) while the microspheres are present in the tissue, applying pulses of ultrasonic energy

to the tissue at an acoustical energy level higher than that required for imaging and at a sufficient energy level to disrupt the microspheres and release the gas bubbles therefrom, the pulses of ultrasonic energy and release of gas bubbles thereby temporarily increasing the porosity of cells in the tissue to facilitate entry of the plasmid into the cells to effect transfection thereof.

Embodiment 15 comprises the method of embodiment 14 wherein the microspheres have an average particle size in the range of about 0.5 to about 20 micrometers.

Embodiment 16 comprises the method of embodiment 14 or embodiment 15 wherein the gas comprises a fluorocarbon gas.

Embodiment 17 comprises the method of any one of embodiments 14 to 16 wherein the shells comprise human serum albumin.

Embodiment 18 comprises the method of any one of embodiments 14 to 17 wherein the active form of ABCA1 has the amino acid sequence of SEQ ID NO: 1.

Embodiment 19 comprises the method of any one of embodiments 14 to 18 wherein the at least one sequence adapted to promote expression of the open reading frame comprises a cytomegalovirus promoter.

Embodiment 20 comprises the method of any one of embodiments 14 to 19 wherein the plasmid is administered in an aqueous carrier at a concentration in the range of about 0.5 to about 50 mg/mL.

Embodiment 21 comprises the method of any one of embodiments 14 to 20 wherein the microspheres are administered in an aqueous carrier at a concentration in the range of about 10^8 to about 10^9 microspheres per milliliter.

Embodiment 22 comprises the method of any one of embodiments 14 to 21 wherein the plasmid and the microspheres are administered as a mixture in one aqueous carrier.

Embodiment 23 comprises the method of any one of embodiments 14 to 21 wherein the plasmid and the microspheres are administered in separate aqueous carriers.

Embodiment 24 comprises the method of any one of embodiments 14 to 23 wherein an additional biologically active agent is co-administered along with the plasmid and microspheres.

Embodiment 25 comprises the method of embodiment 24 wherein the additional biologically active agent comprises at least one material selected from the group consisting of (a) a drug for treating a condition relating to lipid metabolism or transport, (b) a plasmid encoding a protein other than ABCA1 involved in lipid metabolism of transport, and (c) a plasmid encoding an siRNA that targets a protein involved in lipid metabolism or transport.

Embodiment 26 comprises the method of any one of embodiments 14 to 25 wherein the tissue comprises liver tissue, intestinal parenchymal tissue, or a combination thereof. Embodiment 27 comprises use of a composition of any one of embodiments 1 to 13 to enhance high density lipoprotein cholesterol in the blood of a subject (e.g., to treat atherosclerosis).

Embodiment 28 comprises use of a composition of any one of embodiments 1 to 13 for the preparation of a medicament to enhance high density lipoprotein cholesterol in the blood of a subject (e.g., to treat atherosclerosis).

In a study conducted with rats, sonoporation of the liver in conjunction with a peripheral (tail vein) intravenous infusion of an aqueous composition containing ABCA1 plasmids and albumin-encapsulated fluorocarbon gas (octafluoropropane) microspheres resulted in a significant increase in plasma HDL-c levels relative to the baseline HDL-c levels for the rats. In this study, ABCA1 plasmid achieved a higher serum

concentration of HDL-c than apoA1 plasmid. This finding is both surprising and novel the current understanding in this field is the most effective method to increase hepatic secretion of HDL-c is to increase apoA1 synthesis. In fact, treatment with both the ABCA1 plasmid and the apoA1 plasmid was no better than an infusion of ABCA1 alone in this study.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 provides the amino acid sequence of human ABCA1 (SEQ ID NO: 1) utilized in the examples herein.

FIG. 2 provides the open reading frame nucleotide sequence of human ABCA1 (SEQ ID NO: 1) utilized in the examples herein.

FIG. 3 illustrates the nucleotide sequence of the plasmid (SEQ ID NO: 3) utilized in the examples herein.

FIG. 4 provides a schematic illustration of the ABCA1 plasmid utilized in the examples described herein.

FIG. 5 provides a graph of blood HDL-c in rats following sonochemical treatment with compositions comprising OPTISON microspheres and plasmids encoding ABCA1, apoA1, or a combination thereof.

DETAILED DESCRIPTION OF SELECTED EMBODIMENTS

In one aspect, the present invention provides compositions useful for transfecting cells to express ABCA1 comprise a pharmaceutically acceptable aqueous carrier containing sonochemically-active microspheres together with a plasmid DNA construct encoding an active form of ABCA1 and at least one promoter for the expression thereof. The sonochemically-active microspheres comprise, consist essentially of, or consist of gas bubbles encapsulated within protein-containing or lipid-containing shells. The microspheres are disruptable by sonication (i.e., exposure to ultrasonic acoustic energy), releasing the encapsulated gas.

In another aspect, the present invention provides a method of inducing ABCA1 expression in cells *in vivo*, and a method enhancing high density lipoprotein cholesterol in the blood. These methods comprise intravenously co-administering an ABCA1 plasmid and sonochemically active microspheres as described herein to a subject, ultrasonically imaging the a target tissue (e.g., the liver or intestinal parenchymal tissue) of the subject to detect when the microspheres are circulating through the vasculature of the tissue. The imaging typically is performed by external application of ultrasonic acoustic energy at a mechanical index (MI; which is defined as the peak negative acoustic pressure divided by the square of the imaging frequency) less than about 0.4 MI. When the microspheres are detected in the target tissue, pulses of ultrasonic acoustic energy are applied to the tissue at an acoustic energy level higher than the energy level needed for imaging (typically greater than 1 MI, preferably greater than 1.3 MI, and up to about 2 MI), and of sufficiently high acoustic energy to disrupt the shells of the microspheres and release the gas bubbles encapsulated by the shells. The pulses preferably are applied at an acoustic frequency of about 1 to about 7 MHz. The acoustic energy of the pulses and the release of gas bubbles in the tissue temporarily increases the porosity of the tissue cell membranes (a process referred to herein as “sonoporation”).

Sonoporation of a tissue such as the liver comprises supplying ultrasonic acoustic energy pulses to the tissue with the ultrasound imaging probe. The pulses are applied while ultrasonically imaging the tissue so that the pulses are applied primarily when the microspheres (and thus also the co-ad-

ministered plasmid) are present. The pulses typically are applied at a rate of about 6 to about 8 pulses per minute for a total of about 5 to about 20 pulses. The pulse duration typically is in the range of about 500 to about 2000 milliseconds per pulse. The acoustic energy pulses disrupt the microspheres and release the encapsulated gas. This disruption, combined with directly supplied acoustic energy makes the cells more porous to the plasmids, so that the plasmids can enter and transfect the cells. Once transfected, the cells transcribe protein via messenger RNA leading to synthesis of active ABCA1, which in turn enhances HDL formation and HDL-c concentration in the blood.

As used herein, the term “co-administration” and grammatical variations thereof, refers to administering two or more materials to the same individual during the same therapeutic session. Such co-administration can involve administering material separately, or together within the same composition. The co-administration can be simultaneous or can be temporally separated. In addition, sites of co-administration can be in the same location or different locations.

As used herein, the term “plasmid” and grammatical variations thereof refers to small circular DNA that is physically separate from, and can replicate independently of, chromosomal DNA within a cell (i.e., in an episome), and which commonly are found as small (e.g., about one thousand to about one million base pairs) circular, double-stranded DNA molecules in bacteria. Artificial plasmids are used as vectors in molecular cloning, serving to drive the replication of recombinant DNA sequences within episomes in the host cells without altering the chromosomal DNA of the host cells.

As used herein, the term “episomally transfected” and grammatical variations thereof refer to non-insertional (non-integrating) transfection with exogenous episomal nucleic acid, such as DNA, siRNA, RNA, or mRNA (e.g. a plasmid or other episomal vector) to produce a cell with unaltered chromosomal DNA, in which the a polypeptide encoded by the episomal DNA is expressed within the target cells (e.g., liver cells) without genomic integration of the exogenous DNA. As used herein, the term “episome” and grammatical variations thereof refers to closed circular DNA molecules that are replicated in the nucleus, and is intended to encompass exogenous plasmids introduced into host cells such as liver cells. Preferably, the plasmid encodes the active form of ABCA1 and also encodes regulatory elements (e.g., a promoter) to facilitate episomal expression of the ABCA1 protein.

As used herein, the term “active form of ABCA1” and grammatical variations thereof refers to the ABCA1 protein of SEQ ID NO: 1 and variations thereof comprising conservative substitutions in SEQ ID NO: 1 and sharing at least about 95 percent sequence identity (e.g., at least about 95, 96, 97, 98, or 99% sequence identity) with SEQ ID NO: 1 and retaining the lysine residues at positions 939 and 1952 of SEQ ID NO: 1.

Percentage values stated herein are on a weight-weight basis (i.e., “weight percent” or “percent by weight”) when referring to a concentration, and on a number basis when referring to a quantity or countable number of items, as the context will make evident and unless otherwise specified.

As used herein, a “therapeutically effective dosage” is an amount (e.g., a total of about 0.5 to about 10 mL of a single composition or co-administered compositions comprising about 0.5 to about 50 mg/mL of the ABCA1 plasmid and about 10⁸ to about 10⁹ of the microspheres per milliliter) such that when administered in conjunction with sonication of the liver, the plasmids transfect cells of the tissue to express the nucleic acid, subsequently resulting in enhanced HDL-c level in the blood, or other effects targeted by the therapy. The

dosage and number of doses (e.g. single or multiple dose) administered to a subject will vary depending upon a variety of factors, including the route of administration, patient conditions and characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired, the concentration of plasmids in the composition, and the like.

Adjustment and manipulation of dosage ranges, as well as in vitro and in vivo methods of determining the therapeutic effectiveness of the composition in an individual, are well within the ability of those of ordinary skill in the medical arts. In some preferred embodiments, the dosage does not exceed about 5 mL of composition over a 10 minute period. Suitable safe dosages of ultrasonic contrast agents provide a useful guideline for use in the methods described herein. Such safe dosages are well known in the art and are documented in literature from ultrasonic contrast agent manufacturers.

The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

Sonochemically-active microspheres suitable for use in the compositions and methods described herein include any protein-based or lipid-based gas-filled microspheres (also known as "microbubbles") that can be used as ultrasonic imaging contrast agents. The microspheres comprise a physiologically acceptable gas (e.g., a non-toxic gas such as nitrogen, air, oxygen, argon, or a fluorocarbon such as octafluoropropane (also known as "perflutren") or perfluorohexane. The shell of the microspheres can comprise a protein (e.g., human serum albumin), a lipid material (e.g., phospholipids, phosphocholine lipids, and polyethoxylates thereof), or a combination thereof. Some lipid-based microspheres may also contain galactose in the shell. Protein-containing microspheres can include relatively small amounts (e.g., less than about 1 percent) of fatty acids (e.g., caprylic acid), amino acids or amino acid derivatives (e.g., N-acetyltryptophan), or other formulation aids. The microspheres typically have a mean particle size (i.e., effective mean diameter) in the range of about 1 to about 10 micrometers (preferably about 1 to about 5 micrometers). Preferably, at least about 95% of the microspheres have a diameter of less than about 10 micrometers. The microspheres preferably are present in the composition at a concentration in the range of about 10^8 to about 10^9 of the microspheres per milliliter. The compositions described herein preferably are prepared by simple mixing of a microsphere suspension (e.g., as supplied by the manufacturer) with a solution of the plasmid or plasmids that are to be co-administered.

The pharmaceutically active aqueous carrier of the compositions comprises water (e.g., deionized, pyrogen-free water), and preferably includes one or more salts (e.g., sodium chloride, phosphate, citrate, and the like). In some 5 preferred embodiments the aqueous carrier comprises physiological saline (about 0.9 percent NaCl), phosphate buffered saline, and the like. The carrier optionally can include other soluble materials (e.g., dextrose), preservatives, and the like, to provide a solution that is generally sterile, safe, isotonic 10 and compatible with blood. Preferably, the compositions have a physiological pH (e.g., about pH 6.4 to 7.5).

Some preferred microsphere formulations useful in the 15 compositions and methods described herein include the OPTISON brand microspheres (available from GE Healthcare), IMAGENT brand microspheres (developed by Alliance Pharmaceutical), and DEFINITY brand microspheres (available from Lantheus Medical Imaging, Inc.). Preferably, the compositions comprise human serum albumen encapsulated 20 octafluoropropane microspheres, such as OPTISON microspheres.

According to the manufacturer, OPTISON microsphere suspensions from GE Healthcare comprise about 5×10^8 to about 8×10^8 microspheres per mL of suspension. The microspheres comprise perflutren (octafluoropropane) gas bubbles encapsulated within shells of human serum albumin. The microspheres have a mean particle diameter of about 3 to about 4.5 μm with about 95% of the microspheres having a diameter of less than about 10 μm . The microspheres are suspended in a 25 physiological saline solution (about 0.9 percent by weight NaCl in water). The compositions also can include less than about 1 percent caprylic acid, and less than about 1 percent N-acetyltryptophan. Each milliliter of OPTISON microspheres reportedly comprises about 10 mg of human serum albumin, about 0.2 to 0.3 mg of perflutren, about 0.2 mg N-acetyltryptophan, and about 0.12 mg caprylic acid in 0.9% aqueous 30 sodium chloride at a pH of about 6.4-7.4. The headspace of the vial containing the suspension is filled with perflutren gas. The manufacturer recommends that the injection rate should not exceed about 1 mL per second (maximum total dose 35 should not exceed about 5 mL in any 10 minute period, and maximum total dose should not exceed about 8.7 mL in any one patient study).

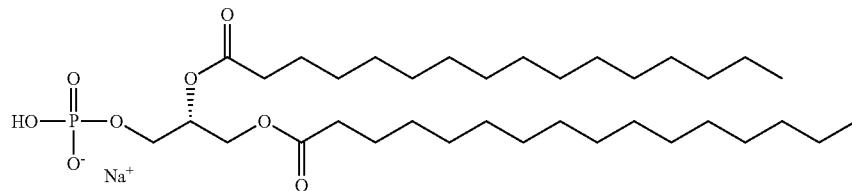
IMAGENT perflexane lipid microsphere composition (trade name previously IMAVIST) is an injectable suspension 45 developed by Alliance Pharmaceutical. The microspheres reportedly comprise perflexane (perfluorohexane) microbubbles encapsulated in a lipid-based shell comprising 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), hydroxyethyl starch and poloxamer (a nonionic triblock copolymer composed of a central hydrophobic chain of polyoxypropylene flanked by two hydrophilic chains of polyoxyethylene). The microspheres are suspended in a phosphate buffered saline solution.

According to manufacturer information, DEFINITY per 50 flutren lipid microspheres are provided as an injectable suspension. The DEFINITY material is supplied as components that upon activation yield perflutren lipid microspheres. The material is supplied in a vial containing a clear, colorless, sterile, non-pyrogenic, hypertonic liquid, which upon activation with the aid of a VIALMIX brand activator, provides a 55 homogeneous, opaque, milky white injectable suspension of perflutren lipid microspheres. The suspension of activated DEFINITY microspheres is administered by intravenous injection. The perflutren lipid microspheres are composed of 60 octafluoropropane encapsulated in an outer lipid shell consisting of (R)-hexadecanoic acid-1-[(phosphonoxy)methyl]-1,2-ethanediyl ester, monosodium salt (abbreviated. DPPA);

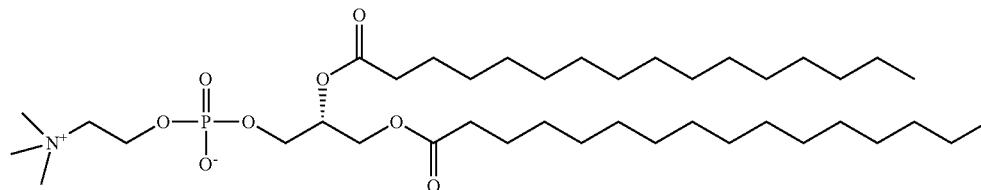
9

(R)-4-hydroxy-N,N,N-trimethyl10-oxo-7-[(1-oxohexadecyl)oxy]-3,4,9-trioxa-4-phosphapentacosan-1-aminium-4-oxide inner salt (abbreviated DPPC); and (R)- α -[6-hydroxy-6-oxido-9-[(1-oxohexadecyl)oxy]5,7,11-trioxa-2-aza-6-phosphahexacos-1-yl]- ω -methoxypoly(ox-1,2-ethanediyl), monosodium salt (abbreviated MPEG5000 DPPE).

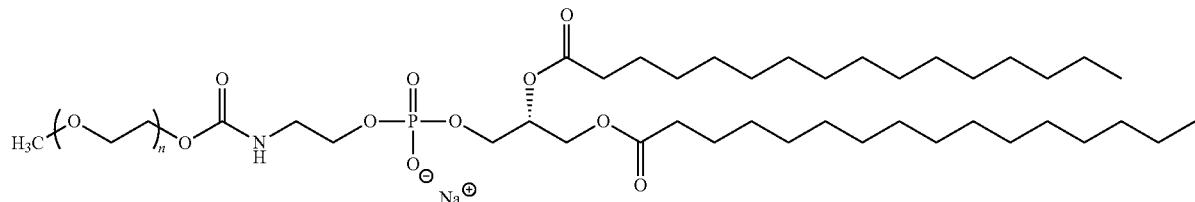
DPPA has a molecular weight of 670, empirical formula of C₃₅H₆₈O₈PNa, and following structural formula:



DPPC has a molecular weight of 734, empirical formula of C₄₀H₈₀NO₈P, and following structural formula:



MPEG5000 DPPE has an approximate molecular weight of 5750 represented by empirical formula C₂₆₅H₅₂₇NO₁₂₃PNa, and the following structural formula:



Prior to VIALMIX activation, the DEFINITY component vial reportedly contains 6.52 mg/mL octafluoropropane in the headspace. Each mL of the clear liquid reportedly contains 0.75 mg lipid blend (consisting of 0.045 mg DPPA, 0.401 mg DPPC, and 0.304 mg MPEG5000 DPPE), 103.5 mg propylene glycol, 126.2 mg glycerin, 2.34 mg sodium phosphate monobasic monohydrate, 2.16 mg sodium phosphate dibasic heptahydrate, and 4.87 mg sodium chloride in water (pH is 6.2-6.8). After activating the contents of the vial, each mL of the milky white suspension reportedly contains a maximum of 1.2 \times 10¹⁰ perflutren lipid microspheres, and about 150 μ m/mL (1.1 mg/mL) octafluoropropane. The microsphere mean particle size is about 1.1 μ m to 3.3 μ m, with 98% of the microspheres having a diameter of less than 10 μ m.

Plasmid Design.

Plasmids generally comprise a strong viral promoter to drive the in vivo transcription and translation of the encoded gene (or complementary DNA, RNA, siRNA, or mRNA) of interest (which is present as an open reading frame). Intron A may can be included to improve mRNA stability and hence

10

increase protein expression. Plasmids also typically include a strong polyadenylation/transcriptional termination signal, such as bovine growth hormone or rabbit beta-globulin polyadenylation sequences.

Because the plasmid provided the genetic material from which the protein of interest is expressed, optimizing vector design for maximal protein expression is desirable. For example, the codon usage can be adjusted to better conform to

eukaryotic cells. Another factor to consider is the choice of promoter. Examples of promoters include the simian virus 40

(SV40) promoter, the Rous Sarcoma Virus (RSV) promoter, and the cytomegalovirus (CMV) promoter. In addition, expression rates can sometimes be improved by inclusion of

enhancer sequences, adenovirus tripartite leader (TPL) sequences, or modifications to the polyadenylation and transcriptional termination sequences. Non-limiting examples of episomal plasmid vectors suitable for use as vectors for transfection of liver cells include SV40-based vectors, Epstein-Barr virus-based vectors, papilloma virus-based vectors, BK virus-based vectors, and the like, which are well known in the molecular genetics art.

Non limiting examples of episomal vectors suitable for use as non-integrating vectors for transfection of eukaryotic cells (e.g., primary MSC) include simian virus 40-based vectors, Epstein-Barr virus-based vectors, papilloma virus-based vectors, BK virus-based vectors, and the like, which are well known in the molecular genetics art.

In some embodiments, an additional biologically active agent is co-administered along with the plasmid and microspheres. Such additional biologically active agents include, for examples, a drug for treating a condition relating to lipid metabolism or transport, a plasmid encoding a protein other than ABCA1 involved in lipid metabolism or transport, and a

11

plasmid encoding an siRNA that targets a protein involved in lipid metabolism or transport. Additionally, or alternatively, the plasmid encoding the active ABCA1 can also encode an a protein other than ABCA1 involved in lipid metabolism of transport, or a siRNA that targets a protein involved in lipid metabolism or transport.

Non-limiting examples of drugs for treating a condition relating to lipid metabolism or transport include HMG-CoA inhibitors, such as statins (e.g., atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, and combinations thereof); bile acid inhibitors (e.g., cholestyramine, colestipol, coleselvalam, and combinations thereof); fibric acid derivatives (e.g., clofibrate, gemfibrozil, fenofibrate) which may lower LDL-c and raise HDL-c; and niacin.

Examples of proteins involved in lipid metabolism or transport include, e.g., ABCA1, ABCG1, apoA1, apoA2, apoE, LXR, NPC1, NPC2, SREBP, CD36, ACAT, SRA1, and HMG-CoA. Such enzymes or siRNA targeting such proteins may be encoded by a plasmid co-administered with the ABCA1 plasmid, or in some cases can be encoded the same plasmid as the ABCA1.

Co-administration of a drug or a plasmid encoding a lipid metabolism/transport protein or siRNA can have the advantage of tailoring treatment to the specific condition suffered by the patient, by invoking multiple action sites, multiple metabolic targets, or both. For example, the materials to be co-administered can be selected e.g., to HDL-c and lower LDL-c in patients where both types of cholesterol are outside the recommended levels, or to raise HDL-c and lower total triglycerides, etc. In addition, different tissues may be transfected in a given subject, e.g., to target optimal tissues that may be involved in the particular lipid metabolic pathways of interest. For example, one tissue may be transfected to express one protein or siRNA, while a different tissue of the same subject may be transfected to express another protein of siRNA.

The following non-limiting examples are provided to illustrate certain features and aspects of the IP-MSC and methods described herein.

Methods and Procedures.

Male Sprague-Dawley rats (180-250 g) were purchased through Charles River Laboratories, Wilmington, Mass. All animal studies performed in an AAALAC, USDA, and OLAW accredited facility. Rats were housed in sterile cages (Alternative Design Manufacturing & Supply Inc., Siloam Springs, Ark.) and provided ad libitum access to standard commercial feed (Lab Diet; Purina Mills, St. Louis, Mo.) and water. Animals were maintained on a 12-hour:12-hour light: dark cycle, controlled temperature (approximately 24° C.) and controlled humidity (approximately 40%).

ApoA1 DNA Plasmids.

An apoA1 expression plasmid was constructed by subcloning an 804 bp human apoA1 PCR cDNA product into expression vector pMIR0125 (Mirus Bio Corp, Madison, Wis.) containing human apoE hepatic control region (HCR), human ubiquitin C promoter and first intron. The final construct, pMIR0332-HCRUbC-h apoA1 was sequenced and the resulting clone matched the reported human apoA1 sequence. An ABCA1 expression vector was purchased from Origene (ABCA1 (NM_005502) Human cDNA ORF Clone, Cat. No. RC221861). FIG. 1 provides the amino acid sequence of ABCA1 (SEQ ID NO: 1) encoded in the open reading frame of the vector. FIG. 2 provides the nucleotide sequence of the ABCA1 open reading frame (SEQ ID NO: 2) of the vector,

12

while FIG. 3 provides the nucleotide sequence of the entire vector (SEQ ID NO: 3). FIG. 4 provides a schematic map of the ABCA1 plasmid.

Infusion Method.

5 The plasmids (i.e., apoA1 plasmid, ABCA1 plasmid, or a combination of apoA1 and ABCA1 plasmids) were mixed with commercial OPTISON microsphere suspension for injection. Anesthetic: Inhalable gas mixture of oxygen and 1.5-3.0% isoflurane. A warming bed maintained rat body 10 temperature at 37° C. Tail vein injections were performed using a 26 GA ¾-inch catheter. Mixtures: About 1 mL of OPTISON microspheres was mixed with (1) apoA1 DNA plasmid (approximately 8 mg in 1 mL), or (2) ABCA1 plasmid (approximately 7.3 mg in 1 mL), or (3) a combination of 15 apoA1 and ABCA1 plasmids at the same concentrations per mL as used for the individual plasmids.

Plasmid Volume and Concentration of DNA.

All plasmids alone or in combination were mixed with 20 OPTISON microspheres for about 15 to about 30 seconds prior to injection with a 3 mL injection syringe and then co-administered or co-infused. The infusion rate was manually performed at a timed rate of about 2 to 3 mL/minute and the total infusion duration was about 50 to about 70 seconds. 25 Ultrasound Equipment and Imaging and Therapeutic Parameters.

A VIVID I brand imaging system (GE Healthcare Systems, Milwaukee, Wis.) equipped with a 3S ultrasound probe was utilized for all liver sonifications. All acoustic energy settings 30 remained within FDA guidelines outlined for diagnostic use (ALARA principle). The rat liver was continually visualized using external ultrasound to verify appearance of OPTISON mixture within the liver vasculature and parenchyma. Low mechanical index (MI) ultrasound acoustic energy was used 35 for imaging (e.g., <0.4 MI) whereas, higher MI (about 1.3 MI or greater) was used for therapy. No surgical abdominal incision was ever performed; external ultrasound was used to visualize the liver. The abdomen was shaved to eliminate surface hair.

40 The ultrasound parameters consisted of an continuous low mechanical index (MI<0.4) ultrasound exposure for "imaging" followed by a two-second "pulse" for therapeutic effect for a total of 10 "pulses." A pulse was defined as a relatively higher (MI>1.3) burst of an ultrasound pulse. In sum, the 45 "pulse" length was about 2 seconds with a pulse interval of about 8 seconds for a total of 10 pulses.

Blood Samples.

Three tail vein blood samples (0.5 ml) were collected over 50 6 days to establish a baseline serum HDL-c for each rat prior to treatment. After treatment with the plasmids in the OPTISON microsphere suspensions, blood samples were collected daily for three days and then after a three day hiatus. All blood samples were collected in glass test tubes containing a lithium heparin anticoagulant. The separated serum samples were 55 analyzed using a clinical lipid panel test strip (PTS #1710 Lipid Panel Test for CARDIOCHEK PA CHOLESTEROL ANALYZER, Polymer Technology Systems, Inc., Indianapolis, Ind.) to quantify HDL values. Graphical data are reported as mean+1 SEM and statistical significance was 60 determined by a two-sample t-test. The null hypothesis was rejected at P<0.05 and all statistical analyses were carried out using MINITAB12 (Minitab Inc., State College, Pa. USA). Results.

FIG. 5 provides a graph of blood HDL-c levels of the 65 treated rats, including the baseline level and levels for three days post-treatment. The selective hepatic transduction of ABCA1 through sonoporation described herein resulted in

13

enhanced blood HDL-c relative to baseline HDL-c concentration. A single sonochemical treatment with an ABCA1 plasmid and sonochemically active microsphere composition in conjunction with sonication of the liver resulted in a 15% increase in HDL-c. A surprising finding in this study was that the ABCA1 plasmid therapy resulted in superior increases in HDL-c relative to treatment with an apoA1 plasmid or the combination of ABCA1 and apoA1 plasmids under the same sonication conditions.

Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred

14

embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 3

<210> SEQ ID NO 1
<211> LENGTH: 2261
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: Human ABCA1

<400> SEQUENCE: 1

Met	Ala	Cys	Trp	Pro	Gln	Leu	Arg	Leu	Leu	Leu	Trp	Lys	Asn	Leu	Thr
1					5			10				15			
Phe	Arg	Arg	Arg	Gln	Thr	Cys	Gln	Leu	Leu	Leu	Glu	Val	Ala	Trp	Pro
	20					25					30				
Leu	Phe	Ile	Phe	Leu	Ile	Leu	Ile	Ser	Val	Arg	Leu	Ser	Tyr	Pro	Pro
	35					40				45					
Tyr	Glu	Gln	His	Glu	Cys	His	Phe	Pro	Asn	Lys	Ala	Met	Pro	Ser	Ala
	50					55				60					
Gly	Thr	Leu	Pro	Trp	Val	Gln	Gly	Ile	Ile	Cys	Asn	Ala	Asn	Asn	Pro
	65				70				75			80			
Cys	Phe	Arg	Tyr	Pro	Thr	Pro	Gly	Glu	Ala	Pro	Gly	Val	Val	Gly	Asn
	85					90				95					
Phe	Asn	Lys	Ser	Ile	Val	Ala	Arg	Leu	Phe	Ser	Asp	Ala	Arg	Arg	Leu
	100					105				110					
Leu	Leu	Tyr	Ser	Gln	Lys	Asp	Thr	Ser	Met	Lys	Asp	Met	Arg	Lys	Val
	115					120				125					
Leu	Arg	Thr	Leu	Gln	Gln	Ile	Lys	Lys	Ser	Ser	Ser	Asn	Leu	Lys	Leu
	130					135				140					
Gln	Asp	Phe	Leu	Val	Asp	Asn	Glu	Thr	Phe	Ser	Gly	Phe	Leu	Tyr	His
	145				150				155			160			
Asn	Leu	Ser	Leu	Pro	Lys	Ser	Thr	Val	Asp	Lys	Met	Leu	Arg	Ala	Asp
	165					170				175					
Val	Ile	Leu	His	Lys	Val	Phe	Leu	Gln	Gly	Tyr	Gln	Leu	His	Leu	Thr
	180					185				190					
Ser	Leu	Cys	Asn	Gly	Ser	Lys	Ser	Glu	Glu	Met	Ile	Gln	Leu	Gly	Asp
	195					200				205					
Gln	Glu	Val	Ser	Glu	Leu	Cys	Gly	Leu	Pro	Arg	Glu	Lys	Leu	Ala	Ala
	210				215				220						
Ala	Glu	Arg	Val	Leu	Arg	Ser	Asn	Met	Asp	Ile	Leu	Lys	Pro	Ile	Leu
	225				230				235			240			
Arg	Thr	Leu	Asn	Ser	Thr	Ser	Pro	Phe	Pro	Ser	Lys	Glu	Leu	Ala	Glu
	245					250				255					
Ala	Thr	Lys	Thr	Leu	Leu	His	Ser	Leu	Gly	Thr	Leu	Ala	Gln	Glu	Leu
	260				265				270						

US 9,101,745 B2

15**16**

-continued

Phe Ser Met Arg Ser Trp Ser Asp Met Arg Gln Glu Val Met Phe Leu
275 280 285

Thr Asn Val Asn Ser Ser Ser Ser Thr Gln Ile Tyr Gln Ala Val
290 295 300

Ser Arg Ile Val Cys Gly His Pro Glu Gly Gly Leu Lys Ile Lys
305 310 315 320

Ser Leu Asn Trp Tyr Glu Asp Asn Asn Tyr Lys Ala Leu Phe Gly Gly
325 330 335

Asn Gly Thr Glu Glu Asp Ala Glu Thr Phe Tyr Asp Asn Ser Thr Thr
340 345 350

Pro Tyr Cys Asn Asp Leu Met Lys Asn Leu Glu Ser Ser Pro Leu Ser
355 360 365

Arg Ile Ile Trp Lys Ala Leu Lys Pro Leu Leu Val Gly Lys Ile Leu
370 375 380

Tyr Thr Pro Asp Thr Pro Ala Thr Arg Gln Val Met Ala Glu Val Asn
385 390 395 400

Lys Thr Phe Gln Glu Leu Ala Val Phe His Asp Leu Glu Gly Met Trp
405 410 415

Glu Glu Leu Ser Pro Lys Ile Trp Thr Phe Met Glu Asn Ser Gln Glu
420 425 430

Met Asp Leu Val Arg Met Leu Leu Asp Ser Arg Asp Asn Asp His Phe
435 440 445

Trp Glu Gln Gln Leu Asp Gly Leu Asp Trp Thr Ala Gln Asp Ile Val
450 455 460

Ala Phe Leu Ala Lys His Pro Glu Asp Val Gln Ser Ser Asn Gly Ser
465 470 475 480

Val Tyr Thr Trp Arg Glu Ala Phe Asn Glu Thr Asn Gln Ala Ile Arg
485 490 495

Thr Ile Ser Arg Phe Met Glu Cys Val Asn Leu Asn Lys Leu Glu Pro
500 505 510

Ile Ala Thr Glu Val Trp Leu Ile Asn Lys Ser Met Glu Leu Leu Asp
515 520 525

Glu Arg Lys Phe Trp Ala Gly Ile Val Phe Thr Gly Ile Thr Pro Gly
530 535 540

Ser Ile Glu Leu Pro His His Val Lys Tyr Lys Ile Arg Met Asp Ile
545 550 555 560

Asp Asn Val Glu Arg Thr Asn Lys Ile Lys Asp Gly Tyr Trp Asp Pro
565 570 575

Gly Pro Arg Ala Asp Pro Phe Glu Asp Met Arg Tyr Val Trp Gly Gly
580 585 590

Phe Ala Tyr Leu Gln Asp Val Val Glu Gln Ala Ile Ile Arg Val Leu
595 600 605

Thr Gly Thr Glu Lys Lys Thr Gly Val Tyr Met Gln Gln Met Pro Tyr
610 615 620

Pro Cys Tyr Val Asp Asp Ile Phe Leu Arg Val Met Ser Arg Ser Met
625 630 635 640

Pro Leu Phe Met Thr Leu Ala Trp Ile Tyr Ser Val Ala Val Ile Ile
645 650 655

Lys Gly Ile Val Tyr Glu Lys Glu Ala Arg Leu Lys Glu Thr Met Arg
660 665 670

Ile Met Gly Leu Asp Asn Ser Ile Leu Trp Phe Ser Trp Phe Ile Ser
675 680 685

US 9,101,745 B2

17**18**

-continued

Ser Leu Ile Pro Leu Leu Val Ser Ala Gly Leu Leu Val Val Ile Leu
 690 695 700

 Lys Leu Gly Asn Leu Leu Pro Tyr Ser Asp Pro Ser Val Val Phe Val
 705 710 715 720

 Phe Leu Ser Val Phe Ala Val Val Thr Ile Leu Gln Cys Phe Leu Ile
 725 730 735

 Ser Thr Leu Phe Ser Arg Ala Asn Leu Ala Ala Ala Cys Gly Gly Ile
 740 745 750

 Ile Tyr Phe Thr Leu Tyr Leu Pro Tyr Val Leu Cys Val Ala Trp Gln
 755 760 765

 Asp Tyr Val Gly Phe Thr Leu Lys Ile Phe Ala Ser Leu Leu Ser Pro
 770 775 780

 Val Ala Phe Gly Phe Gly Cys Glu Tyr Phe Ala Leu Phe Glu Glu Gln
 785 790 795 800

 Gly Ile Gly Val Gln Trp Asp Asn Leu Phe Glu Ser Pro Val Glu Glu
 805 810 815

 Asp Gly Phe Asn Leu Thr Thr Ser Val Ser Met Met Leu Phe Asp Thr
 820 825 830

 Phe Leu Tyr Gly Val Met Thr Trp Tyr Ile Glu Ala Val Phe Pro Gly
 835 840 845

 Gln Tyr Gly Ile Pro Arg Pro Trp Tyr Phe Pro Cys Thr Lys Ser Tyr
 850 855 860

 Trp Phe Gly Glu Glu Ser Asp Glu Lys Ser His Pro Gly Ser Asn Gln
 865 870 875 880

 Lys Arg Ile Ser Glu Ile Cys Met Glu Glu Pro Thr His Leu Lys
 885 890 895

 Leu Gly Val Ser Ile Gln Asn Leu Val Lys Val Tyr Arg Asp Gly Met
 900 905 910

 Lys Val Ala Val Asp Gly Leu Ala Leu Asn Phe Tyr Glu Gly Gln Ile
 915 920 925

 Thr Ser Phe Leu Gly His Asn Gly Ala Gly Lys Thr Thr Thr Met Ser
 930 935 940

 Ile Leu Thr Gly Leu Phe Pro Pro Thr Ser Gly Thr Ala Tyr Ile Leu
 945 950 955 960

 Gly Lys Asp Ile Arg Ser Glu Met Ser Thr Ile Arg Gln Asn Leu Gly
 965 970 975

 Val Cys Pro Gln His Asn Val Leu Phe Asp Met Leu Thr Val Glu Glu
 980 985 990

 His Ile Trp Phe Tyr Ala Arg Leu Lys Gly Leu Ser Glu Lys His Val
 995 1000 1005

 Lys Ala Glu Met Glu Gln Met Ala Leu Asp Val Gly Leu Pro Ser
 1010 1015 1020

 Ser Lys Leu Lys Ser Lys Thr Ser Gln Leu Ser Gly Gly Met Gln
 1025 1030 1035

 Arg Lys Leu Ser Val Ala Leu Ala Phe Val Gly Gly Ser Lys Val
 1040 1045 1050

 Val Ile Leu Asp Glu Pro Thr Ala Gly Val Asp Pro Tyr Ser Arg
 1055 1060 1065

 Arg Gly Ile Trp Glu Leu Leu Leu Lys Tyr Arg Gln Gly Arg Thr
 1070 1075 1080

 Ile Ile Leu Ser Thr His His Met Asp Glu Ala Asp Val Leu Gly
 1085 1090 1095

US 9,101,745 B2

19

20

-continued

Asp Arg Ile Ala Ile Ile Ser His Gly Lys Leu Cys Cys Val Gly
 1100 1105 1110
 Ser Ser Leu Phe Leu Lys Asn Gln Leu Gly Thr Gly Tyr Tyr Leu
 1115 1120 1125
 Thr Leu Val Lys Lys Asp Val Glu Ser Ser Leu Ser Ser Cys Arg
 1130 1135 1140
 Asn Ser Ser Ser Thr Val Ser Tyr Leu Lys Glu Asp Ser Val
 1145 1150 1155
 Ser Gln Ser Ser Ser Asp Ala Gly Leu Gly Ser Asp His Glu Ser
 1160 1165 1170
 Asp Thr Leu Thr Ile Asp Val Ser Ala Ile Ser Asn Leu Ile Arg
 1175 1180 1185
 Lys His Val Ser Glu Ala Arg Leu Val Glu Asp Ile Gly His Glu
 1190 1195 1200
 Leu Thr Tyr Val Leu Pro Tyr Glu Ala Ala Lys Glu Gly Ala Phe
 1205 1210 1215
 Val Glu Leu Phe His Glu Ile Asp Asp Arg Leu Ser Asp Leu Gly
 1220 1225 1230
 Ile Ser Ser Tyr Gly Ile Ser Glu Thr Thr Leu Glu Glu Ile Phe
 1235 1240 1245
 Leu Lys Val Ala Glu Glu Ser Gly Val Asp Ala Glu Thr Ser Asp
 1250 1255 1260
 Gly Thr Leu Pro Ala Arg Arg Asn Arg Arg Ala Phe Gly Asp Lys
 1265 1270 1275
 Gln Ser Cys Leu Arg Pro Phe Thr Glu Asp Asp Ala Ala Asp Pro
 1280 1285 1290
 Asn Asp Ser Asp Ile Asp Pro Glu Ser Arg Glu Thr Asp Leu Leu
 1295 1300 1305
 Ser Gly Met Asp Gly Lys Gly Ser Tyr Gln Val Lys Gly Trp Lys
 1310 1315 1320
 Leu Thr Gln Gln Gln Phe Val Ala Leu Leu Trp Lys Arg Leu Leu
 1325 1330 1335
 Ile Ala Arg Arg Ser Arg Lys Gly Phe Phe Ala Gln Ile Val Leu
 1340 1345 1350
 Pro Ala Val Phe Val Cys Ile Ala Leu Val Phe Ser Leu Ile Val
 1355 1360 1365
 Pro Pro Phe Gly Lys Tyr Pro Ser Leu Glu Leu Gln Pro Trp Met
 1370 1375 1380
 Tyr Asn Glu Gln Tyr Thr Phe Val Ser Asn Asp Ala Pro Glu Asp
 1385 1390 1395
 Thr Gly Thr Leu Glu Leu Leu Asn Ala Leu Thr Lys Asp Pro Gly
 1400 1405 1410
 Phe Gly Thr Arg Cys Met Glu Gly Asn Pro Ile Pro Asp Thr Pro
 1415 1420 1425
 Cys Gln Ala Gly Glu Glu Glu Trp Thr Thr Ala Pro Val Pro Gln
 1430 1435 1440
 Thr Ile Met Asp Leu Phe Gln Asn Gly Asn Trp Thr Met Gln Asn
 1445 1450 1455
 Pro Ser Pro Ala Cys Gln Cys Ser Ser Asp Lys Ile Lys Lys Met
 1460 1465 1470
 Leu Pro Val Cys Pro Pro Gly Ala Gly Gly Leu Pro Pro Pro Gln
 1475 1480 1485

-continued

Arg Lys Gln Asn Thr Ala Asp Ile Leu Gln Asp Leu Thr Gly Arg
 1490 1495 1500
 Asn Ile Ser Asp Tyr Leu Val Lys Thr Tyr Val Gln Ile Ile Ala
 1505 1510 1515
 Lys Ser Leu Lys Asn Lys Ile Trp Val Asn Glu Phe Arg Tyr Gly
 1520 1525 1530
 Gly Phe Ser Leu Gly Val Ser Asn Thr Gln Ala Leu Pro Pro Ser
 1535 1540 1545
 Gln Glu Val Asn Asp Ala Ile Lys Gln Met Lys Lys His Leu Lys
 1550 1555 1560
 Leu Ala Lys Asp Ser Ser Ala Asp Arg Phe Leu Asn Ser Leu Gly
 1565 1570 1575
 Arg Phe Met Thr Gly Leu Asp Thr Lys Asn Asn Val Lys Val Trp
 1580 1585 1590
 Phe Asn Asn Lys Gly Trp His Ala Ile Ser Ser Phe Leu Asn Val
 1595 1600 1605
 Ile Asn Asn Ala Ile Leu Arg Ala Asn Leu Gln Lys Gly Glu Asn
 1610 1615 1620
 Pro Ser His Tyr Gly Ile Thr Ala Phe Asn His Pro Leu Asn Leu
 1625 1630 1635
 Thr Lys Gln Gln Leu Ser Glu Val Ala Leu Met Thr Thr Ser Val
 1640 1645 1650
 Asp Val Leu Val Ser Ile Cys Val Ile Phe Ala Met Ser Phe Val
 1655 1660 1665
 Pro Ala Ser Phe Val Val Phe Leu Ile Gln Glu Arg Val Ser Lys
 1670 1675 1680
 Ala Lys His Leu Gln Phe Ile Ser Gly Val Lys Pro Val Ile Tyr
 1685 1690 1695
 Trp Leu Ser Asn Phe Val Trp Asp Met Cys Asn Tyr Val Val Pro
 1700 1705 1710
 Ala Thr Leu Val Ile Ile Phe Ile Cys Phe Gln Gln Lys Ser
 1715 1720 1725
 Tyr Val Ser Ser Thr Asn Leu Pro Val Leu Ala Leu Leu Leu
 1730 1735 1740
 Leu Tyr Gly Trp Ser Ile Thr Pro Leu Met Tyr Pro Ala Ser Phe
 1745 1750 1755
 Val Phe Lys Ile Pro Ser Thr Ala Tyr Val Val Leu Thr Ser Val
 1760 1765 1770
 Asn Leu Phe Ile Gly Ile Asn Gly Ser Val Ala Thr Phe Val Leu
 1775 1780 1785
 Glu Leu Phe Thr Asp Asn Lys Leu Asn Asn Ile Asn Asp Ile Leu
 1790 1795 1800
 Lys Ser Val Phe Leu Ile Phe Pro His Phe Cys Leu Gly Arg Gly
 1805 1810 1815
 Leu Ile Asp Met Val Lys Asn Gln Ala Met Ala Asp Ala Leu Glu
 1820 1825 1830
 Arg Phe Gly Glu Asn Arg Phe Val Ser Pro Leu Ser Trp Asp Leu
 1835 1840 1845
 Val Gly Arg Asn Leu Phe Ala Met Ala Val Glu Gly Val Val Phe
 1850 1855 1860
 Phe Leu Ile Thr Val Leu Ile Gln Tyr Arg Phe Phe Ile Arg Pro
 1865 1870 1875

-continued

-continued

<210> SEQ ID NO 2
<211> LENGTH: 6783
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ABCA1 Open Reading Frame
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: ABCA1 Open Reading Frame

<400> SEQUENCE: 2

atggcttgtt ggccctagct gaggttgcgt ctgtgaaaga acctcacttt cagaagaaga 60
caaacatgtc agctgctgct ggaagtggcc tggcctctat ttatcttcct gatectgtatc 120
tctgttccggc tgagctaccc accctatgaa caacatgaat gccatttcc aaataaagcc 180
atgccctctg caggaacact tccttgggtt caggggatta tctgtaatgc caacaacccc 240
tgttcccggtt acccgactcc tggggaggtt cccggagttt ttggaaactt taacaaatcc 300
attgtggctc gcctgttctc agatgctcg aggcttctt tatacagcca gaaagacacc 360
agcatgaagg acatgctgcaaa agttctgaga acattacagc agatcaagaa atccagctca 420
aacattgaagc ttcaagattt cctgggtggac aatgaaaacct tctctgggtt cctgtatcac 480
aacctctctc tcccaaagtc tactgtggac aagatgctga gggctgatgt catttccac 540
aaggataaaa ttgcaaggcata ccagttacat ttgacaagtc tttgtcaatgg atcaaaatca 600
gaagagatga ttcaacttgg tgaccaagaa gtttctgagc tttgtggctt accaaggag 660
aaactggctg cagcagagcg agtacttcgt tccaaacatgg acatccgtt gccaatccctg 720
agaacactaa actctacatc tcccttcccg agcaaggagc tggctgaagc cacaaaaada 780
ttgctgcata gtcttggac tctggccag gagcttgcata gcatgagaag ctggagtgcac 840
atgcgacagg aggtgatgtt tctgaccaat gtgaacagct ccagctctc cacccaaatc 900
taccaggctg tgcgtcgat tgcgtcgccg catcccgagg gaggggggct gaagatcaag 960
tctctcaact ggtatgagga caacaactac aaagccctct ttggaggcaa tggcactgag 1020
gaagatgctg aaaccttcta tgacaaactct acaactctt actgcaatga tttgtatgaag 1080
aatttggagt ctagtccctt ttcccgatt atctggaaag ctctgaagcc gctgtcgat 1140
gggaagatcc tgtatacacc tgacactcca gcccacaaggc aggtcatggc tgagggtgaac 1200
aagacccctcc aggaactggc tgcgttccat gatctggaaag gcatgtgggaa ggaactcagc 1260
cccaagatct ggacccctat ggagaacagc caagaaaatgg accttgcgtt gatgtgttg 1320
gacagcaggc acaatgacca ctttggaa cagcagttgg atggctttaga ttggacagcc 1380
caagacatcg tggcggtttt ggcaagcac ccagaggatg tccagttccag taatggttct 1440
gtgtacacctt ggagagaagc tttcaacagc actaaccagg caatccggac cataatctcg 1500
ttcatggagt gtgtcaacctt gaacaagcttta gaacccatag caacagaatgt ctggctcatc 1560
aacaagtccca tggagatgtt ggtatgaggg aagttctggg ctggatgtt gttactggaa 1620
attactccag gcacgattgtt gctgccccat catgtcaagt acaagatccg aatggacatt 1680
gacaatgtgg agaggacaaa taaaatcaag gatgggtact gggaccctgg tcctcgatgt 1740
gacccttttggaggcgttccat gtacgatgttgggggtttcg cctacttgca ggtatgtgggt 1800
gacagcaggcata tcatcgatgtt gctgacgggc accgagaaga aactgggtt ctatatgca 1860
cagatgcctt atccctgttta cgatgttgc acatcttgc ggtatgtt ggggtcaatgt 1920
ccccctttca tgacgatgttgc ctggatttac tcaatgggttgc tgatcatcaa gggcatcgatc 1980
tatgagaagg aggcacgggtt gaaagagacc atgcggatca tggggcttggaa caacacatcg 2040

-continued

ctctggttta gctggttcat tagtagcctc attccctttc ttgtgagcgc tggcctgcta	2100
gtggtcatcc tgaaggtagg aaacctgctg ccctacagt atcccagcgt ggtgtttgtc	2160
ttccctgtccg tgtttgcgt ggtgacaatc ctgcagtgc tctgtattag cacactttc	2220
tccagagcca acctggcagc agcctgtggg ggcataatc acttcacgct gtacctgcc	2280
tacgtctgt gtgtggcatg gcaggactac gtgggctca cactcaagat ctgcgtac	2340
ctgctgtctc ctgtggctt tgggttggc tgtgagttt ttgccttt tgaggagcag	2400
ggcattggag tgcagtggga caacctgttt gagagtcctg tggaggaaga tggcttaat	2460
ctcaccaccc cggctccat gatgctgtt gacaccttc tctatgggt gatgacctgg	2520
tacattgagg ctgttttcc aggccagttc ggaatttcca ggccctggta tttcccttgc	2580
accaagtcc actgggttgg cgaggaaagt gatgagaaga gccaccctgg ttccaaccag	2640
aagagaatat cagaaatctg catggaggag gaacccaccc acttgaagct gggcggttgc	2700
attcagaacc tggtaaaagt ctaccgagat gggatgaagg tggctgtcga tggctggca	2760
ctgaattttt atgaggggca gatcaccttc ttccctggcc acaatggagc ggggaagacg	2820
accacatgt caatcctgac cgggttgttc cccccgaccc cgggcaccgc ctacatctg	2880
ggaaaagaca ttgcgtctga gatgagcacc atccggcaga acctgggggt ctgtccccag	2940
cataacgtgc tggttgcacat gctgactgtc gaagaacaca tctggttcta tgccgcctt	3000
aaagggtct ctgagaagca cgtgaaggcg gagatggagc agatggccct ggtgttgg	3060
ttgcccataa gcaagctgaa aagcaaaaaca agccagctgt cagggtggaaat gcagagaaag	3120
ctatctgtgg ccttggccctt tgccggggga tctaagggtt tcattctgga tgaacccaca	3180
gctgggtgtt acccttactc ccgcaggggg atatggagc tgctgtgaa ataccgacaa	3240
ggccgcacca ttattctctc tacacaccac atggatgaag cggacgtcct gggggacagg	3300
attgccatca tctccatgg gaagctgtgc tgggtggct cctccctgtt tctgaagaac	3360
cagctggaa caggctacta cctgaccttg gtcaagaaat atgtggaaat ctcctcagt	3420
tcctgcagaa acagtagtag cactgtgtca tacctgaaaa aggaggacag tggttctcag	3480
agcagttctg atgctggcct gggcagcgcac catgagatgt acacgctgac catcgatgtc	3540
tetgttatct ccaacactcat caggaagcat gtgtctgaag cccggctgtt ggaagacata	3600
gggcatgagc tgacctatgt gctgccccat gaagctgtca aggaggaggc ctttggaa	3660
ctcttcatg agattgtatga cccgctctca gacctggca tttctagttt tggcatctca	3720
gagacgaccc tggaaagaaat attcctcaag gtggccgaag agagtgggggt ggtgtctgag	3780
acctcagatg gtaccttgcc agcaagacga aacaggcggg ctttggggga caagcagac	3840
tgtcttcgccc cgttactgtt agatgtatgt gctgatccaa atgattctga catagaccca	3900
gaatccagag agacagactt gctcagtgaa atggatggca aagggttctt ccaggtgaaa	3960
ggctggaaac ttacacagca acatgggttgc gcccctttgtt ggaagagact gcttaatttgc	4020
agacggagtc gggaaaggatt ttttgcgtcatttgc cagctgtgtt tggctgtcatt	4080
gccttgcgtt tcaagctgtat cgtgcaccc tttggcaagt accccagcct ggaacttcag	4140
ccctggatgt acaacgaaca gtacacattt gtcagcaatg atgctctgtt ggacacggga	4200
accctggaaac tcttaaacgc cctcacccaa gaccctggct tcgggaccgc ctgtatggaa	4260
ggaaacccaa tcccagacac gcccgtccag gcaggggagg aagagtggac cactgccccca	4320
gttccccaga ccatcatggc cctcttccag aatggaaact ggacaatgca gaacccttca	4380
cctgcgtgcc agtgttagcag cgacaaaatc aagaagatgc tgcgtgttgc tccccaggg	4440

-continued

gcaggggggc tgcctcctcc acaaagaaaa caaaacactg cagatatcct tcaggacctg	4500
acaggaagaa acatccgga ttatctggtg aagacgtatg tgcagatcat agccaaaagc	4560
ttaaagaaca agatctgggt gaatgagttt aggtatggcg gctttccct gggtgtcagt	4620
aataactcaag cacttcctcc gagtcaagaa gttaatgatg ccatcaaaca aatgaagaaa	4680
cacctaagc tggccaagga cagttctgca gatcgattt tcaacagctt gggagattt	4740
atgacaggac tggacaccaa aaataatgtc aagggtgtgg tcaataacaa gggctggcat	4800
gcaatcagct ctccctgaa tgtcatcaac aatgccattt tccgggccaa cctgcaaaag	4860
ggagagaacc ctagecatta tggattact gcttcaatc atcccctgaa tctcaccaag	4920
cagcagctct cagaggtggc tctgatgacc acatcagtgg atgtccctgt gtccatctgt	4980
gtcatcttc caatgtcctt cgccccagcc agcttgcgtc tattccgtat ccaggagcgg	5040
gtcagcaaag caaaacacct gcagttcatc agtggagtga agcctgtcat ctactggctc	5100
tctaattttg tctggatata tgcattttt gttgtccctg ccacactggt cattatcatc	5160
ttcatctgt tccagcagaa gtcctatgtg tccctccacca atctgcctgt gctagccctt	5220
ctactttgc tgtatgggtg gtcaatcaca cctctcatgt acccagccctc ctttgcgttc	5280
aagatccccca gcacagccta tgggtgcgc accagcgtga acctcttcat tggcattaat	5340
ggcagcgtgg ccaccttgc gctggagctg ttcaccgaca ataagctgaa taatataat	5400
gatatacctga agtccgtgtt cttgatcttcc ccacatcccc gcctgggacg agggctcatc	5460
gacatggtga aaaaccaggc aatggctgtat gcccctggaa ggtttgggaa gaatgcctt	5520
gtgtcaccat tatcttggga cttgggtggga cggaaacctct tcgccccatggc cgtggaaagg	5580
gtgggtttct tcctcattttc tggtctgtatc cagtagatgtat tcttcatttc gcccagaccc	5640
gttaaatgcata agctatctcc tctgaatgtat gaagatgaag atgtggggcg ggaaagacag	5700
agaattcttgc atgggtggagg ccagaatgac atcttagaaaa tcaaggagtt gacgaagata	5760
tatagaagga agcggaaagcc tgctgttgc accatggcgtgg tggccattttc tcctgggtgag	5820
tgctttgggc tcctgggagt taatggggct ggaaaatcat caacttccaa gatgttaaca	5880
ggagatacca ctgttaccag aggagatgtt tcccttaaca aaaatgtat cttatcaaacc	5940
atccatgaag tacatcagaa catgggttac tggccatgtt ttgtatgcattt cacagagctg	6000
ttgactggga gagaacacgt ggagttctt gccccttgc gaggagtcgg agagaagaa	6060
gttggcaagg ttgggtgatgt ggccgattcgaa aaactggggcc tcgtgaagta tggagaaaa	6120
tatgtggta actataatgtgg aggcaacaaaa cggaaacctct ctacagccat ggctttgtatc	6180
ggccggccctc ctgtgggtttt tctggatgaa cccaccacag gcatggatcc caaagcccg	6240
cgggtttttgtt ggaattgtgc cctaaatgtttt gtcaaggagg ggagatcaat agtgcattaca	6300
tctcatagta tggaaatgt tgaagcttt tgcacttagga tggcaatcat ggtcaatggaa	6360
aggttcagggtt gccttggcag tgcattttttt ctaaaaaata ggtttgggaa tggttatata	6420
atagttgtac gaatagcagg gtccaaacccg gacctgaagc ctgtccaggaa tttctttggaa	6480
cttgcatttc ctggaaatgtt tctaaatgtt aaacaccggc acatgttaca ataccagctt	6540
ccatcttcattt tatcttctctt ggccaggata ttcagcatcc tctcccttgc caaaagcga	6600
ctccacatag aagactactc tgtttcttagt acaacacttg accaagtatt tgcactttttt	6660
gccaaggacc aaaaatgtatgtt tgaccactta aaagacccatc cattacacaa aaaccagaca	6720
gtatgtggacg ttgcattttt cttttttttt ctacaggatg agaaatgtaa agaaatgtat	6780
gtatgtggacg ttgcattttt cttttttttt ctacaggatg agaaatgtaa agaaatgtat	6783

-continued

```

<210> SEQ_ID NO 3
<211> LENGTH: 11675
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ABCA1 plasmid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: ABCA1 plasmid

<400> SEQUENCE: 3

aacaatata taacgcttac aattccatt cgccattcag gtcgcgaac tggggaaag      60
ggcgatcggt gcggccctct tcgctattac gccagctggc gaaaggggga tggctgcaaa    120
ggcgattaag ttggtaacg ccagggtttt cccagtcacg acgttgtaaa acgacggcca    180
gtgccaagct gatctataca ttgatcaat attggcaatt agccatatta gtcattggtt    240
atatacgata aatcaatatt ggctattggc cattgcatac gttgtatcta tatcataata    300
tgtacattta tattggctca tgtccatat gaccgccatg ttgacattga ttattgacta    360
gttattaata gtaatcaatt acggggcat tagttcatag cccatatatg gagttcccg      420
ttacataact tacggtaaat ggccgcctg gctgaccgc caacgacccc cgcccatgt     480
cgtaataat gacgtatgtt cccatagtaa cgccaatagg gactttccat tgacgtcaat    540
gggtggagta tttacggtaa actgcccact tggcagtaca tcaagtgtat catatgcca     600
gtccgcggcc tattgacgta aatgccccgc ctggcattat gcccagtaca    660
tgaccttacg ggacttccct acttggcagt acatctacgt attagtcatc gctattacca    720
tggtgatgcg gtttggcag tacaccaatg ggcgtggata gcggttgac tcacgggat     780
ttccaagtct ccacccatt gacgtcaatg ggagttgtt ttggcaccaa aatcaacggg    840
actttccaaa atgtcgtaat aaccccgccc cggtgacgca aatggggcgtt aggcggtac    900
ggtgggaggt ctatataagc agagctcggt tagtgaaccg tcagaatttt gtaatacgac    960
tcactatagg gggccggga attcgctgac tggatccggt accgaggaga tctggcccg     1020
cgatcgccat ggcttggcgt cctcagctga gggtgctgt gtggagaac ctcactttca   1080
gaagaagaca aacatgtcag ctgctgctgg aagtggcctg gctctatatt atcttctga   1140
tcctgatctc tggcgctg agctacccac cctatgaaca acatgaatgc cattttcaa   1200
ataaagccat gccccttgca ggaacacttc cttgggttca ggggattatc tggatgcca   1260
acaacccctg tttccgttac ccgactcctg gggaggctcc cggagttgtt ggaaacttta  1320
acaatccat tggcgctgc ctgttctcgat atgctcgag gcttcttta tacagccaga   1380
aagacaccag catgaaggac atgcgcaag ttctgagaac attacagcag atcaagaat    1440
ccagctaaa ctgaaaggctt caagatcc tggcgacaa tggaaaccttc tctgggttcc   1500
tgtatcacaat cttctctctc cccaaagtcta ctgtggacaa gatgctgagg gctgatgtca 1560
ttctccacaat ggtatccatg caaggctacc agttacattt gacaagtctg tgcaatggat 1620
caaaatcaga agagatgatt caacttggtg accaagaatg ttctgagctt tggccctac  1680
caaggggaa actggctgca gcagagcggag tacttcgttc caacatggac atcctgaagc  1740
caatcctgag aacactaac tctacatctc cttcccgag caaggagctg gctgaagcca  1800
caaaaacatt gctgcatagt cttgggactc tggcccagga gctgttcagc atgagaagct  1860
ggagtgcacat ggcacaggag gtgtatgttca tgaccaatgtt gaacagctcc agctctcca 1920
cccaaatcta ccaggctgtt tctcgatattt tctgcgggca tcccgaggaa ggggggctga  1980
agatcaagtc tctcaactgg tatgaggaca acaactacaa agccctctt ggaggcaatg  2040

```

-continued

gcactgagga agatgctgaa acttctatg acaactctac aactcattac tgcaatgatt	2100
tgatgaagaa ttggagtct agtctcttt cccgattat ctggaaagct ctgaaggcgc	2160
tgctcggtgg gaagatcctg tatacacctg acactccagc cacaaggcag gtcattgt	2220
aggtgaacaa gaccccttccag gaactggctg tggatcatg tctggaaaggc atgtgggagg	2280
aactcagccc caagatctgg accttcatgg agaacagcca agaaatggac cttgtccgga	2340
tgctgttggc cagcaggac aatgaccact ttgggaaaca gcagttggat ggcttagatt	2400
ggacagccca agacatcgtg gctgttttgg ccaagcaccc agaggatgtc cagtcagta	2460
atggttctgt gtacacctgg agagaagctt tcaacgagac taaccaggca atccggacca	2520
tatctcgctt catggagtgt gtcaacactg acaagctaga accccatagca acagaagtct	2580
ggctcatcaa caagtccatg gagctgtgg atgagaggaa gttctgggtt ggtatttgt	2640
tcactggaat tactccaggc agcattgagc tgccccatca tgtcaagtac aagatccgaa	2700
tgacatttga caatgtggag aggacaaata aaatcaagga tgggtactgg gaccctggc	2760
cctcgagctga cccctttttag gacatgcggg acgtctgggg gggcttgcgg tacttgcagg	2820
atgtggtggc gcaggcaatc atcagggtgc tgacgggcac cgagaagaaa actgggtct	2880
atatgcaaca gatgccctat ccctgttacg ttgatgacat ctttctgcgg gtgatgagcc	2940
ggtcaatgcc cctcttcatg acgctggcct ggatttactc agtggctgtg atcatcaagg	3000
gcatcgtgttga tgagaaggag gcacggctga aagagaccat gcggatcatg ggcctggaca	3060
acagcatctt ctggtttagc tggttcatta gtggctcat tcctcttgcgtg gtgagcgt	3120
gcctgctagt ggtcatcctg aagtttagaa acctgctgcc ctacagtgtat cccagcgtgg	3180
tgtttgtctt cctgtccgtg tttgtgtgg tgacaatctc gcagtgttcc ctgattagca	3240
cactcttc cagagccaaac ctggcagcagc cctgtggggg catcatctac ttcacgcgt	3300
acctgcctta cgtccgtgtgt gtggcatggc aggactacgt gggcttccaca ctcaagatct	3360
tcgctagccct gctgtctccct gtggcttttg gttttggctg tgagttactt gccccttttgc	3420
aggagcaggg cattggagtg cagtgggaca acctgtttga gagtcctgtg gaggaagatg	3480
gcttcatatc caccacttcg gtctccatga tgctgtttga caccccttc tatgggtgt	3540
tgacctggta cattggggct gtctttccag gccagttacgg aattccagg ccctggatt	3600
ttccctgcac caagtcctac tggttggcg aggaaagtga tgagaagagc caccctgggt	3660
ccaaaccagaa gagaatatca gaaatctgca tggaggagga acccaccac ttgaagctgg	3720
gctgtccat tcagaacctg gtaaaagtct accgagatgg gatgaaggctg gctgtcgatg	3780
gectggcaact gaatttttat gaggggcaga tcacctcctt cctggccac aatggagcgg	3840
ggaagacgac caccatgtca atcctgaccg gttgttccc cccgacactcg ggcacccct	3900
acatccctggg aaaagacatt cgctctgaga tgagcaccat cggcagaac ctgggggtct	3960
gtcccccagca taacgtgttg tttgacatgc tgactgtcgaa agaacacatc tggttctatg	4020
cccgcttgaa agggctctc gagaagcagc tgaaggcgg gatggagcag atggccctgg	4080
atgtttggttt gccatcaagc aagctgaaaa gcaaaacaag ccagctgtca ggtggaaatgc	4140
agagaaagct atctgtggcc ttggcccttg tcgggggatc taaggttgc attctggatg	4200
aacccacagc tgggtggac ctttactccc gcagggaaat atgggagctg ctgctgaaat	4260
accgacaagg cccgaccatt attctctcta cacaccatc ggttgcggc gacgtccctgg	4320
gggacaggat tgccatcatc tcccatggaa agctgtgttg tggggctcc tccctgttcc	4380
tgaagaacca gctggaaaca ggctactacc tgacccttggt caagaaagat gtgaaatct	4440

-continued

ccctcagttc ctgcagaaac agtagtagca ctgtgtcata cctgaaaaag gaggacagt	4500
tttctcagag cagttctgat gctggcctgg gcagcgacca tgagagtgac acgctgacca	4560
tcatgtctc tgctatctcc aacctcatca ggaagcatgt gtctgaagcc cggctggtgg	4620
aagacatagg gcatgagctg acctatgtgc tgccatatga agctgtaag gaggagcct	4680
ttgtgaaact ctttcatgag attgtatgacc ggctctcaga cctggcatt tctagttatg	4740
gcatctcaga gacgaccctg gaagaatatat tcctcaagggt ggccgaagag agtggggtgg	4800
atgctgagac ctcagatggt accttgccag caagacgaaa caggcgggcc ttccccgaca	4860
agcagagctg ttttcgccc ttcactgaag atgatgtgc tgatccaaat gattctgaca	4920
tagacccaga atccagagag acagacttgc tcagtggtt ggtggcaaa ggttcctacc	4980
aggtgaaagg ctggaaactt acacagcaac agtttgccg cctttgtgg aagagactgc	5040
taattgccag acggagtcgg aaaggatttt ttgctcagat tgtcttgcca gctgtgttg	5100
tctgcattgc ctttgttgc acgctgatcg tgccaccctt tggcaagtac cccagctgg	5160
aacttcagcc ctggatgtac aacgaacagt acacatttg cagcaatgat gctcctgagg	5220
acacggaaac cctggaaactc tttaacgccc tcaccaaaga ccctggctc gggacccgct	5280
gtatgaaagg aaacccaatc ccagacacgc cctgcccaggc agggggaggaa gagtggacca	5340
ctgccccagt tccccagacc atcatggacc tcttccagaa tggaaactgg acaatgcaga	5400
acccttcacc tgcattgcag tgtagcagcg acaaatcaa gaagatgctg cctgtgtgc	5460
ccccagggcc aggggggctg cctctccac aaagaaaaca aaacactgca gatacccttc	5520
aggacctgac aggaagaaac atttcggatt atctgggtaa gacgtatgtg cagatcatag	5580
ccaaaagctt aaagaacaag atctgggtga atgagtttag gtatggccgc tttccctgg	5640
gtgtcagtaa tactcaagca ctccctccga gtcagaagt taatgtgcc atcaaacaaa	5700
tgaagaaaca cctaaagctg gccaggaca gttctgcaga tcgattctc aacagctgg	5760
gaagatttat gacaggactg gacacaaaa ataatgtcaa ggtgtggtgc aataacaagg	5820
gtggcatgc aatcagctct ttccctgaatg tcatcaacaa tgccattctc cgggccaacc	5880
tgcääaaagg agagaaccct agccattatg gaattactgc ttcaatcat cccctgaatc	5940
tcaccaagca gcagctctca gaggtggctc tgatgaccac atcagtggat gtcctgtgt	6000
ccatctgtgt catcttgca atgccttgc tcccagccg ctttgcgtt ttcctgatcc	6060
aggagccgggt cagcaaagca aaacacctgc agttcatcag tggagtgaag cctgtcatct	6120
actggctctc taatttgtc tgggatatgt gcaattacgt tgccctgcc acactggtca	6180
ttatcatctt catctgcttc cagcagaagt cctatgtgtc ctccaccaat ctgcctgtgc	6240
tagcccttc acttttgctg tatgggtgtt caatcacacc ttcattgtac ccagectct	6300
ttgtgttcaa gatccccaggc acagccatcg tggtgctcac cagcgtgaac ctcttcattg	6360
gcattaatgg cagcgtggcc acctttgtgc tggagctgtt caccgacaat aagctgaata	6420
atatcaatga tatcctgaag tccgtgttct tgatcttccc acattttgc ctggacgag	6480
ggctcatcga catggtgaaa aaccaggca tggctgtatgc cctggaaagg tttggggaga	6540
atcgctttgt gtcaccatta tcttggact tggtgggacg aaacctcttc gccatggccg	6600
tggaaagggt ggtgttcttc ctcattactg ttctgatcca gtacagattc ttcattcaggc	6660
ccagacactgt aatgcaaag ctatctctc tgaatgtatg agatgaagat gtgaggccgg	6720
aaagacagag aattcttgcat ggtggaggcc agaatgacat cttagaatc aaggagtgt	6780
cgaagatata tagaaggaag cgaaaggctg ctgttgacag gatttgcgtg ggcattctc	6840

-continued

ctgggtgagtg ctttgggctc ctgggagtt aatggggctgg aaaatcatca actttcaaga	6900
tgttaaacagg agataccact gttaccagag gagatgtttt ccttaacaaa aatagtatct	6960
tatcaaacat ccatgaagta catcagaaca tgggctactg ccctcagtt gatgecatca	7020
cagagctgtt gactgggaga gaacacgtgg agttcttgc cctttgaga ggagtcccag	7080
agaaagaagt tggcaagggtt ggtgagtggtt cgattcgaa actgggcctc gtgaagtatg	7140
gagaaaaata tgctggtaac tatagtggag gcaacaaacg caagctctc acagecatgg	7200
cttgatcg cgggcctctt gtgggtttc tggatgaacc caccacaggc atggatccca	7260
aagccccggc gttcttgtgg aatttgtgcc taagtgtgtt caaggagggg agatcagtag	7320
tgcttacatc tcatagtatg gaagaatgtg aagcttttg cactaggatg gcaatcatgg	7380
tcaatggaaag gtcaggtgc cttggcagtg tccagcatct aaaaaatagg tttggagatg	7440
gttatacaat agttgtacga atagcagggtt ccaacccggc cctgaagcct gtccaggatt	7500
tctttggact tgcatttcctt ggaagtgttc taaaagagaa acaccggaaac atgctacaat	7560
accagcttcc atcttcattt atcctctctgg ccaggatatt cagcatccctc tcccagagca	7620
aaaagcgact ccacatagaa gactactctg tttctcagac aacacttgac caagtatttgc	7680
tgaactttgc caaggaccaa agtgatgtg accacttaaa agacctctca ttacacaaaa	7740
accagacagt agtggacgtt gcagttctca catctttct acaggatgag aaagtgaaag	7800
aaagctatgt aacgcgtacg cggccgctcg agcagaaaact catctcagaa gaggatctgg	7860
cagcaaatga tatactggat tacaaggatg acgacgataa ggtttaacgg gccggccgc	7920
gtcatagctg tttcctgaac agatcccccgg tggcatccct gtgaccctc cccagtgcct	7980
ctcctggccc tggaaagttgc cactccagtg cccaccagcc ttgtcctaattt aaaaatatttgc	8040
tgcatttgcattt ttttgcattt ggtgtccttc tataatattt tgggggtggag ggggggtgtt	8100
tggagcaagg ggcaagttgg gaagacaacc tggtagggctt ggggggtctt ttggaaacca	8160
agctggatgtt cagtggcaca atcttggctc actgcaatctt ccgcctccgtt ggttcaagcg	8220
attcttgcattt ttcagctcc cgagttgttgg ggattccagg catgcatgac caggctcagc	8280
taattttgtt tttttgtt ggggggtt ttcaccatattt tggccaggctt ggttcaac	8340
tccaatctc aggtgatcta cccaccttgg cttccaaat tggatggattt acaggcgatgt	8400
accactgtctc cttccctgt cttctgtt taaaataac tataccagca ggaggacgtc	8460
cagacacagc ataggctacc tggccatgcc caaccgggtt gacatttgatgtt tggatgtt	8520
ggcaactgtcc tctcatgcgt tgggtccact cagtagatgc ctgttgaattt gggatcgcc	8580
ccagcggcga gcggtatcag ctcactcaaa ggcggtaata cgggttatcca cagaatcagg	8640
ggataacgcgaa gggaaagaaaca tggtagccaa aggccagcaaa aaggccagga accgtaaaa	8700
ggccggcggtt tgggggtttt tccataggctt cggccccctt gacggatc acaaaaatcg	8760
acgctcaagt cagagggtggc gaaaccggac aggactataaa agataccagg cgtttccccc	8820
tggaaagctcc ctcgtgcgtt cttctgttcc gaccctggcc cttaccggat acctgtccgc	8880
cttctccctt tcggaaagcg tggcgcttcc tcatagtctca cgctgttaggtt atctcagttc	8940
gggtgttagtc gttcgcttca agctgggctg tggtagccaa ccccccgttc agcccgaccg	9000
ctgcgcctta tccggtaact atcgttgcgtt gtcacccgg gtaagacacg acttacgtcc	9060
actggcggca gcaactggta acaggattag cagagcgagg tatgttagggcgtt gatctgcgc	9120
gttcttgcgtt tgggtggcttca actacggcttca cactagaaga acagtttttgcgtt gatctgcgc	9180
tctgcttgcgtt ccagtttaccc tccggaaaaag agttggatgtt tttgtatccg gcaaaacaaac	9240

-continued

caccgctgggt	agcggtggtt	tttttgttgc	caagcagcag	attacgcgc	aaaaaaaagg	9300
atctcaagaa	gatccttga	tctttctac	ggggctcgac	gctcagtgg	acgaaaactc	9360
acgttaaggg	atttggtca	tgagattatc	aaaaaggatc	ttcacctaga	tcctttaaa	9420
ttaaaaatga	agttttaat	caatctaaag	tatatatgag	taacctgagg	ctatggcagg	9480
gcctgcgc	ccgacgttgg	ctgcgagcc	tgggcctca	cccgaaactg	gggggtgggg	9540
tggggaaaag	gaagaaacgc	ggggttattg	gccccatgg	ggtctcggt	gggttatcgac	9600
agagtgcac	ccctgggacc	gaaccccgcg	tttatgaaca	aacgacccaa	caccgtgcgt	9660
tttattctgt	cttttatttgc	ccgtcatagc	gcgggttct	tccgtatgt	tctccttccg	9720
tgtttcagtt	agcctcccc	tagggtggc	gaagaactcc	agcatgagat	ccccgcgtg	9780
gaggatcatc	cagccggcgt	cccgaaaac	gattccgaag	cccaacctt	catagaaggc	9840
ggcggtggaa	tcgaaatctc	gtgatggcag	gttggcgtc	gttgggtcgg	tcatttcgaa	9900
ccccagagtc	ccgctcagaa	gaactcgtca	agaaggcgat	agaaggcgat	gchgctgcgaa	9960
tcgggagcgg	cgataccgta	aagcacgagg	aagcggtcag	cccatcgcc	gccaaagctct	10020
ttagtgcgtc	caacgctatg	tcctgatagc	gatccgcac	acccagccgg	10080	
ccacagtcga	tgaatccaga	aaagcggcca	tttccacca	tgtatattcgg	caagcaggca	10140
tcgccccatgg	tcacgacgag	atcctcgcc	tcggcatgc	tcgccttgc	cctggcgaac	10200
atttcggctg	gchcgagccc	ctgatgcct	tcgtccagat	catcctgatc	gacaagaccg	10260
gtttccatcc	gagtaacgtgc	tcgtcgatg	cgatgttgc	cttgggtgtc	aatggcag	10320
gtagccggat	caagegtatg	cageccgcgc	attgcatcag	ccatgtatgg	tactttctcg	10380
gcaggagcaa	ggtgagatga	caggagatcc	tgcccccggca	cttcgcacaa	tagcagccag	10440
tcccttcccg	cttcaatgc	aacgtcgac	acagctgcgc	aaggAACGCC	cgtcggtggcc	10500
agccacgata	gccgegctgc	ctcgcttgc	agttcattca	gggcacccgg	cagggtcggtc	10560
ttgacaaaaaa	gaacggggcg	ccccgtcgat	gacagccgg	acacggggc	atcagagcag	10620
ccgattgtct	gttggccca	gtcatagccg	aatagcctct	ccacccaaagc	ggccggagaa	10680
cctgcgtgc	atccatcttgc	ttcaatcatg	cgaaacgatc	ctcatactgt	ctcttgcgtc	10740
atctttgcaa	aaggcttaggc	ctccaaaaaa	gcctcctc	tacttctgg	atagctcaga	10800
ggccgaggcg	gcctcgcc	ctgcataaaat	aaaaaaaaatt	agtcagccat	ggggcggaga	10860
atggggcgaa	ctggggcgag	ttagggccgg	gttggccgg	gttagggccg	ggactatgg	10920
tgctgactaa	ttgagatgc	tgctttgc	acttctgc	gttggggagc	ctggggactt	10980
tccacacctg	gttgcgtact	aattgagatg	catgcttgc	atacttctgc	ctgctgggaa	11040
gcctggggac	tttccacacc	ctaactgaca	cacattccac	agctggttct	ttccgcctca	11100
ggactcttcc	ttttcaata	ttattgaac	atttacagg	gttattgtct	catgagccgaa	11160
tacatatttgc	aatgtatttgc	aaaaataaa	caaataagg	ttccgcgcac	atttcccgaa	11220
aaagtgcac	ctgacgcgc	ctgtacgcgc	gcattaagcg	ggcgccgtgt	ggtggttacg	11280
cgcagcgtga	ccgctacact	tgccagcgc	ctagcgc	ctcccttcgc	tttctccct	11340
tcctttctcg	ccacgttgc	cggtttccc	cgtcaagctc	taaatcg	ggcccttta	11400
gggttccat	tttagtgc	acggcac	gacccaaaaa	aacttgat	gggtgtatgg	11460
tcacgtatgc	ggccatcgcc	ctgatagc	gttttcgc	ctttgacgtt	ggagtccacg	11520
ttcttaata	gtggactctt	gttccaaact	ggaacaacac	tcaaccctat	ctcggtctat	11580

-continued

```
tcttttgatt tataagggat tttgccgatt tcggccattt ggtaaaaaa tgagctgatt 11640
taacaaaaat ttaacgcgaa tttaacaaa atatt 11675
```

We claim:

1. A pharmaceutical composition for increasing HDL-cholesterol levels in the blood of a patient, the composition comprising a mixture of a nucleic acid encoding an expressible open reading frame and sonochemically-active microspheres in a pharmaceutically acceptable aqueous carrier; wherein the nucleic acid of the mixture consists of a plasmid vector, the expressible open reading frame consists of an expressible open reading frame encoding the active form of ATP-binding cassette transporter A1 (ABCA1), and the plasmid vector also includes at least one sequence adapted to promote expression of the open reading frame in a mammalian cell; and wherein the sonochemically-active microspheres comprise gas bubbles encapsulated within shells comprising a protein, a lipid, or a combination thereof, the microspheres being disruptable upon exposure to ultrasonic acoustic energy to release the encapsulated gas bubbles; and wherein the composition transfests liver cells when intravenously administered to the patient while ultrasonically imaging the liver, to elicit production of an increased level of HDL-cholesterol in the blood of the patient compared to the HDL-cholesterol level in the blood of the patient prior to administering the composition.

2. The composition of claim **1** wherein the microspheres have an average particle size in the range of about 0.5 to about 20 micrometers.

3. The composition of claim **1** wherein the gas bubbles comprise a fluorocarbon gas.

4. The composition of claim **1** wherein the shells comprise human serum albumin.

5. The composition of claim **1** wherein the active form of ABCA1 has the amino acid sequence of SEQ ID NO: 2.

6. The composition of claim **1** wherein the open reading frame has the nucleotide sequence of SEQ ID NO: 1.

7. The composition of claim **1** wherein the at least one sequence adapted to promote expression of the open reading frame comprises a cytomegalovirus promoter.

8. The composition of claim **1** wherein the plasmid is present in the composition at a concentration in the range of about 0.5 to about 50 milligrams per milliliter.

9. The composition of claim **1** wherein the microspheres are present in the composition at a concentration in the range of about 10^8 to about 10^9 microspheres per milliliter.

10. The composition of claim **1** wherein the aqueous carrier comprises physiological saline, optionally buffered at physiological pH.

11. The composition of claim **1** further comprising a drug for treating a condition relating to lipid metabolism or transport.

12. A pharmaceutical composition for increasing HDL-cholesterol levels in the blood of a patient, the composition comprising a mixture of about 0.5 to about 50 milligrams per milliliter of a nucleic acid encoding an expressible open reading frame and about 108 to about 109 microspheres per milliliter of sonochemically-active microspheres in a pharmaceutically acceptable aqueous carrier; wherein the nucleic acid of the mixture consists of a plasmid vector, the expressible open reading frame consists of an expressible open reading frame encoding the active form of ATP-binding cassette transporter A1 (ABCA1), and the plasmid vector also includes at least one sequence adapted to promote expression of the open reading frame in a mammalian cell; and wherein the sonochemically-active microspheres comprise fluorocarbon gas bubbles encapsulated within shells comprising human serum albumin, the microspheres being disruptable upon exposure to ultrasonic acoustic energy to release the encapsulated gas bubbles; and wherein the composition transfests liver cells when intravenously administered to the patient while ultrasonically imaging the liver, to elicit production of an increased level of HDL-cholesterol in the blood of the patient compared to the HDL-cholesterol level in the blood of the patient prior to administering the composition.

13. The composition of claim **12** wherein the active form of ABCA1 has the amino acid sequence of SEQ ID NO: 2.

14. The composition of claim **12** wherein the open reading frame has the nucleotide sequence of SEQ ID NO: 1.

* * * * *